Metal stress alters a bacterial community's permissiveness towards plasmids

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Characterization of electricity generation and prokaryotic community in plant-microbial fuel cell

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A microcosm study was performed to identify environmental factors and microorganisms affecting the electricity generation of plant-microbial fuel cell (PMFC) using rice plant. The voltage variation showed a diurnal cycle (0.1~0.3 volts with external resistance of 100 Ω) and coincided with the temperature variation (15~39 °C), indicating that temperature is the primary factor affecting electricity generation. The increasing difference in pH between anodic part and cathodic part was also the important factor limiting the power generation. Pyrosequenicng analysis of the 16S rRNAs revealed that the activity of *Deltaproteobacteria* were increased in the anode when the circuit was connected, especially of those related to *Desulfocapsa* and *Geobacter*. One sequence affiliated with *Gammaproteobacteria* occupied 5.3~14.8% of the bacterial 16S rRNAs in the cathode when the circuit was connected but it was not detected when the circuit was unconnected. The portion of methanogens among archaeal 16S rRNAs in the anode was decreased when the circuit was connected, indicating that the installation of microbial fuel cell in sedimnet decreased the activity of methanogens. Bacteria related to the genera *Aeromonas*, *Bacillus*, *Cellulomonas*, *Clostridium*, *Geothrix*, *Opitutus*, *Paenibacillus*, *Rhodopseudomonas*, *Pseudomonas*, *Roseateles*, *Rummeliibacillus* and *Streptomyces* were isolated from the anode and their ability to generate electricity was examined.

High-throughput sequencing of isopycnic centrifugation gradients improves the sensitivity of rRNA-based stable isotope probing

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Stable isotope probing (SIP) of rRNA identifies the microorganism capable of assimilating an isotopically labeled compound in natural environments. The isotope-labeled RNA was separated physically from the unlabeled RNA by isopycnic centrifugation, and then detected by molecular ecological tools such as terminal-restriction fragment length polymorphism (T-RFLP) and clone library sequencing. The resolution in the latter step should influence on the sensitivity of rRNA-SIP. High-throughput sequencing of the rRNA amplicons is available to screen the isotope-incorporating microorganisms at high resolutions, yet its effectiveness and validness remain to be clarified. In this study, we investigated to what extent the sensitivity of rRNA-SIP was improved by the high-throughput illumina sequencing of isopycnic centrifugation gradients. Prior to the experiment, the detection limit of the ^{13}C-labeled RNA by T-RFLP was also evaluated. The ^{13}C-labeled RNA was extracted from pure culture of *Escherichia coli* with ^{13}C-labeled glucose, and the different two unlabeled RNAs were prepared from those of *E. coli* and *Bacillus subtilis* with unlabeled glucose. The ^{13}C-labeled and unlabeled RNAs from *E. coli* were mixed with the unlabeled RNA from *B. subtilis* at ratios from 1% to 0.0001%. These RNA mixtures were density-separated, and then analyzed by T-RFLP and illumina sequencing. In the T-RFLP fingerprints for the ^{13}C mixtures of both 1% and 0.5%, T-RF of *E. coli* was predominated with increase in density and accounted for more than half of the total peak height in the heaviest fraction. The T-RF was slightly detected (i.e., at 6.1% of the total peak height) in the heaviest fraction but not in light

fractions for the ^{13}C mixture of 0.05%, while it could not be detected at all for the 0.01% mixture. In the T-RFLP patterns of the unlabeled mixtures from 1% to 0.05%, no difference was found between heavy and light fractions. These results indicated that T-RFLP detected the ^{13}C-labeled RNA at the mixing ratios at 0.05% as a lower limit. In the illumina-sequencing libraries of the heaviest fractions, the relative abundances of *E. coli* for the ^{13}C mixtures from 0.05% to 0.001% were significantly higher than those for the unlabeled mixtures. The increasing rates were 6.9-, 4.6-, and 3.8-times for the mixtures of 0.05%, 0.01%, and 0.001%, respectively. Meanwhile, the significant increase was not observed in the mixture of 0.0001%. The illumina sequencing of the light fraction represented the comparable abundances of *E. coli* between the ^{13}C and unlabeled mixtures. Furthermore, the enrichment of the ^{13}C-labeled RNA from *E. coli* in the heaviest fractions was confirmed by reverse transcription and quantitative PCR. These results indicated that the illumina sequencing was able to detect the ^{13}C-labeled RNA even at the mixing ratio of 0.001%. Taken together, the high-throughput illumina sequencing of isopycnic centrifugation gradients had much higher sensitivity to detect the ^{13}C-labeled RNA than T-RFLP did, thereby allowing the identification of hitherto-unknown microorganisms involved in greater diversity of biogeochemical processes in natural environments.

Effects of salinity and volatile fatty acids on the growth of an anammox bacterium, *Candidatus* Scalindua sp.

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Anaerobic ammonium oxidation (anammox) is a microbiological process in which ammonium is oxidized to dinitrogen gas under anoxic conditions with nitrite as the electron acceptor. This process is mediated by anammox bacteria belonging to the phylum Planctomycetes. Previous studies have demonstrated the ubiquitous distribution of anammox bacteria in artificial and natural ecosystems such as wastewater treatment plants, freshwater and marine sediments, and soils. *Candidatus* Scalindua is primarily found in marine environments. According to the previous study, *Candidatus* Scalindua sp. have a high nitrogen removal rate as well as freshwater species, and prefer lower temperature than freshwater species. On the other hands, the biomass yield of *Candidatus* Scalindua sp. corresponds to the half value of biomass yield of freshwater species.

In the present study, to clarify what environmental factors affect the biomass yield of *Candidatus* Scalindua sp., the nitrogen removal activity and inorganic carbon uptake activity were measured under different salinity and organic carbon.

The batch experiments were carried out to estimate the biomass yield of marine anammox bacteria under different salinity and formate, acetate, or propionate concentrations. [^{14}C]-labeled bicarbonate was used as a tracer to measure inorganic carbon uptake. The biomass yield was calculated from the nitrogen removal and carbon uptake activities. The phylogenetic analysis based on 16S rRNA gene and fluorescence *in situ* hybridization analysis were conducted to confirm the predominant anammox species and population for the experiments.

Higher carbon uptake was observed at 1.5 to 3.5% salinity. The presence of organic carbon did not affect the anammox activity, carbon uptake, or biomass yield, whereas salinity affected the biomass yield of *Candidatus* Scalindua sp. The results obtained from the present study might contribute new insights into the niche adaptation of marine anammox bacteria.

Bacteria and phage interactions in natural communities

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Bacteria and their viruses (phage) are the most abundant and diverse taxonomic groups, but ecological and evolutionary research on bacteria-phage interactions has largely focused on simplified communities using a few model organisms. We were interested in how bacteria and phage interact within natural environments, and how these interactions are mediated by the surrounding community. We created a gradient of community diversity using serial dilutions of aquatic communities acquired from two different beech tree holes, into which we inoculated a phage-bacteria pair.

I used a *Pseudomonas fluorescens* SBW25 and SBW25φ2 phage to determine if the diversity of the natural bacterial community in which this phage-bacteria pair are introduced affects their coevolution (i.e reciprocal evolution of resistance of the host and infectivity of the phage). I transferred SBW25 and φ2 into dilution cultures of the two tree hole bacterial communities. Bacterial cell density, SBW25 and SBW25φ2 infectivity were monitored for 6 weeks.

The results show little effect of reducing community diversity on coevolutionary dynamics, but the effect of SBW25φ2 on SBW25 abundance differed between communities collected from different sites. This suggests coevolutionary paths might be sensitive to the composition of the bacterial community and that the trajectory of coevolution might depend on the surrounding community.

Studying coevolution within naturally occurring communities in microcosms is valuable as it reveals ecological constraints on coevolution imposed by natural environments.

Low substrate concentration and predator-prey interactions induce rapid adaptations in a freshwater bacterial isolate

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Top-down and bottom-up factors both have a strong impact on bacterial populations in aquatic habitats. Being a major mortality source for aquatic bacteria predation has led to the development of various adaptations that enhance anti-predator fitness. At the same time, microbes that enter into aquatic habitats, e.g., from terrestrial realms, often face substrate-limiting conditions, and predation avoidance mechanisms demand the investment of scarce resources. Here we describe a long-term evolutionary experiment with a freshwater isolate adapted to substrate-rich conditions, *Sphingobium* sp. Z007, and its flagellate predator *Poterioochromonas* sp. The bacterial strain has a predation sensing mechanism which induces enhanced aggregation in the presence of the predator. A co-culture of bacteria and flagellates was maintained by serial propagation for 200 days in an oligotrophic medium and compared to a control treatment featuring bacteria only. Cell densities and bacterial aggregate formation were determined by flow cytometry and isolates were obtained at biweekly intervals. Isolated strains were tested for phenotypic characteristics such as growth kinetics, aggregation, biofilm formation and substrate specificity. Oligotrophic conditions increased growth efficiency of *Sphingobium* in the absence of predation and reduced its ability to form aggregates and biofilms. By contrast, the co-culture led to increased bacterial aggregate and biofilm formation that was ultimately independent of the presence of predators. Furthermore, co-culture experiments with flagellates and isolates from either

treatment showed the loss of the predator sensing response present in the ancestral strain. To obtain a deeper insight into the genotypic base of the phenotypic changes we performed whole genome sequencing of the ancestral and evolved strain. Preliminary evaluation of the sequenced genomes indicated adaptations to both, oligotrophy and predation pressure. In summary, long-term exposure to oligotrophic conditions in combination with or without predation had a strong impact on the growth patterns of *Sphingobium* sp. Z007. Our results moreover indicate that a fluctuating predation regime may be necessary to maintain the ability to sense predators in these bacteria.

Towards predictable manipulation of microbial communities

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Our long-term objective is to gain knowledge about microbial interactions in nature and the human body so as to allow manipulation of microbial communities in a predictable way. Manipulation of these communities is important because they are essential in industrial processes, biogeochemical cycles, and human health. We will begin to tackle this objective by first dissecting microbial interactions in a relatively simple community. High Arctic microbes in Northern Greenland will serve as our model. Specifically, we plan to produce phylogenetic, metagenomic and metatranscriptomic profiles of the community, cultivate key microbial species and produce a set of reference genomes, and integrate this with the “omic” data. These data should allow us to identify what roles specific species play within the community, what molecules mediate key process, and predict how the community will change if these molecules are manipulated. This project is a multi-year interdisciplinary effort that will synergistically combine state-of-the-art “meta-omics” and advanced cultivation tools. Here we report the results of the first year of the project, which focused on microbial cultivation, 16s rRNA gene inventory, and preliminary metatranscriptomic study.

A comprehensive set of reference genomes implies cultivation of the “uncultivable” microbial majority. Thus, we employed several innovated microbial cultivation methodologies in order to gain access to previously uncultured taxa. We used in situ cultivation approaches that allow for exchange of nutrients and growth factors between the cultivation device and the natural environment. Experimental sites included soils, fresh lakes, and marine sediments. Cultivation devices were first incubated in situ to allow indigenous microorganisms to grow under conditions closely mimicking their naturel milieu. Device contents were subsequently subcultured in the lab at 0°C or 10°C to simulate natural conditions. Individual colonies were collected, purified, and archived. In total, about 500 pure cultures of psychrophilic and mesophilic microorganisms were identified by sequencing the 16S rRNA gene (bacteria) or ITS region (yeasts and fungi). The largest diversity was found when samples were cultured in the lab at 10°C.

Soil and sediment samples were also collected from each site for 16s rRNA gene survey and transcriptomic analysis. The marine site was found to be the least diverse, via both culture-dependent and independent approaches; lake samples showed intermediate diversity, and soil samples exhibited the largest number of phylotypes. While 16S rRNA gene and metatranscriptomic analysis are currently underway, the available data identified the lake community as optimal in its richness for further examination. In Year 2 (2014), we will conduct a large scale culture-dependent and meta-omic study of this community as it

changes over time during the short Arctic seasons. By mapping the “omics” results to the reference genomes of cultivated species we aim to learn the roles of individual species in the community as they change over time. We will focus on specific metabolites likely to have significant impacts on microbial dynamics, such as signaling molecules and antibiotics. Modeling the microbial interactions mediated by these molecules should allow us, in Year 3, to manipulate the lake community in a predictable way.

Do pitcher plant species control the assembly of pitcher microbiomes? Differences between bacterial and fungal communities

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Bacteria and fungi are integral components of microbiomes, but the forces structuring the groups may be different. Carnivorous pitcher plants are ideal models for microbial ecology, as the aquatic microcosms within the pitchers are small and self-contained. Pitchers are sterile before opening, and once a pitcher opens it forms an entire food web of arthropods, bacteria, fungi, and protists. The pitcher microbiome is hypothesized to assist the plant in digestion and nutrient acquisition from prey. Plants can lower the acidity of internal fluids by excreting hydrogen ions, and by changing the environment a species may restrict habitat colonization. We collected over one hundred samples from three *Nepenthes* species in three sites in Singapore in 2012 and 2013. Total pitcher volume and pH were recorded in 2013, and soil samples were collected across the sites. We sequenced 16S and 18S rRNA using Illumina MiSeq technology to characterize the prokaryotes and eukaryotes within pitcher microbiomes. Our results indicate pitcher communities are distinct from those in surrounding soil. Multivariate community analyses reveal bacterial community composition is most influenced by pH and host species, while fungal community composition is most influenced by collecting site and year. Host species, site, and pH also structure arthropods and the Stramenopiles, Alveolates, and Rhizaria (SAR) clade of protists, while total volume has no effect on any community. Community matrices of prokaryotes and eukaryotes are significantly correlated, and the organisms are likely interacting via predation, competition, mutualism and parasitism. The different forces shaping the structure of different groups suggest no single principle will explain the community assembly of the pitcher plant microbiome.

Characterization of metabolically active microorganisms in an active hydrothermal field in the Okinawa Trough (IODP Exp. 331)

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The objective of the BGR project, as part of the post- cruise research of IODP Expedition 331, Deep Hot Biosphere, was to test the hypothesis that the quantitative microbial community composition and the cultivable microorganisms in hydrothermally influenced deeply- buried marine sediments are significantly different from those in cold and temperate deeply-buried marine sediments. The previously successfully applied molecular techniques real-time PCR (qPCR) and catalyzed reporter deposition - fluorescence in situ hybridisation (CARD - FISH) have been used as well as cultivation to proof the existence of a deep hot biosphere, to describe it and to isolate novel microorganisms. The domains Archaea, Bacteria and Eukarya as well as the JS1 candidate group, Chloroflexi, Geobacteraceae, Crenarchaeota and the functional genes *dsrA, mcrA, aprA*, and Rubisco (cbbL) have been quantified via qPCR.

All genes have been detected in different copy numbers. The overall order of abundance is Archaea > Bacteria > Eukarya. Directly after IODP Expedition 331 in October 2010, culture media were inoculated with IODP samples at different temperatures and the enrichment cultures are maintained since then. Growth is continuously checked about every three months and in case of growth, colonies are picked and transferred to fresh media. Several aerobic and anaerobic enrichments have been obtained so far. Based on partial 16S rRNA gene sequencing, isolates from the manganese oxidizing enrichment cultures reveal similarity to *Bacillus aquimaris* (94%) and *Bacillus oceani* (93%). Isolates obtained under aerobic conditions with a mix of organic polymeres as carbon source revealed similarities to cultivated species of *Halobacillus litoralis* (92%), *Marinobacter salsuginis* (90%), *Shewanella benthica* (89%) and *Cytophaga fermentans* (92%). Microcalorimetric measurements with the original samples showed a considerable heat production due to exothermic reactions at 90°C which was partly attributed to microbial activity. The microcalorimetric measurements revealed activity of thermophilic microorganisms in the IODP Exp. 331 samples.

Emergence of a synergistic diversity as a response to competition in mixed biofilm

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In natural and man-made environments, biofilms are known to be complex associations of microorganisms which development is controlled by a network of bacterial interactions. As biofilm is a dynamic environment, these interactions evolve over time, often illustrating the adaptation of the whole bacterial community to its environmental conditions on one hand and of the bacterial community members to each other on the other hand. From this process the structure and the functional properties of the whole biofilm finally emerges. Genetic diversification and selection of variants with increased fitness is one of the known mechanisms enabling the adaptation of species to the biofilm way of life. Therefore, a better understanding of the emergence of this genetic diversity and overall the adaptation mechanisms of bacterial species to the biofilm is thus a first step towards the managing of ecosystem functions and evolution.

In this aim, we focused on the adaptation of the model strain *Pseudomonas putida* KT2440 (GFP-tagged) in mixed biofilm with another strain from the same species (*P. putida* PCL1480 mCherry-tagged) more recently isolated from roots of plants. Structural dynamics of mixed biofilms obtained by 3D confocal microscopy demonstrated that KT2440 GFP strain was initially outcompeted by the PCL1480 mCherry strain in the beginning of the dynamic but it was able to adapt after 168h of co-culture. We thus analysed the emergence of colony morphology variants of *P. putida* KT2440 GFP in pure culture or in mixed biofilms with the *P. putida* mCherry strain after this development time. Variants were then phenotypically characterized (growth rate, cell surface properties, swimming, swarming, biofilm architecture) and their genome was fully sequenced (Ion Torrent ®) and analyzed to identify mutations at the origin of the specific variant phenotypes.

Three distinct colony-morphotype of *P. putida* KT2440 GFP were obtained from the mixed biofilm whereas no variant was identified from the axenic *P. putida* KT2440 biofilm. Variants exhibited distinct phenotypes and produced biofilm with specific architecture in comparison with the ancestor. Taken individually, each variant also better competed with the *P. putida* mCherry strain in the mixed biofilms compared with the ancestor after 24h of growth. The whole genome sequencing of variants revealed for instance the presence of mutations in polysaccharides biosynthesis protein, membrane transporter or lipoprotein signal peptidase that provide insights on the molecular origin of the specific phenotypes of the variants. In

addition, we observed that the mix of the three variants and ancestor produced biofilm with high biovolume and had a better ability to compete the *P. putida* PCL1480 mCherry strain than each variant separately, illustrating a synergistical effect of strain association in the adaptation of the whole GFP population. The synergetic interactions between variants supporting the increased fitness of strain KT2440-derived population are discussed in the light of identified mutations.

To sum up, this work provides evidence that an intra-specific competition can foster the emergence of phenotypic diversification which might increase the global fitness of the resulting population and highlights the importance of bacterial interactions in the evolution and adaptation of microbial communities.

Controlled regime shifts in sulphur-cycling microcosms

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Microbial nutrient cycling plays an essential role in biogeochemistry. The microbial sulphur cycle is of particular importance environmentally, and it has been estimated that sulphate reducing bacteria are responsible for up to 50% of the recycling of organic material in marine ecosystems. Understanding the microbial sulphur cycle also has many useful biotechnological applications, from bioremediation, to anaerobic wastewater treatment. However, despite the importance of this cycle, the dynamics and structure of the associated microbial community, as well its complex interactions with the geochemistry of the environment, remain poorly understood. Consequently, the performance of many important technologies relying on the microbial sulphur cycle (such as bioreactors) remain unpredictable. Furthermore, nutrient-cycling microbial ecosystems are mainly studied *in situ*, making it difficult to apply well-controlled perturbations.

We have used sulphur-cycling freshwater pond sediment microbial microcosms (Winogradsky columns) as a controlled experimental model in which to study the effect of perturbations on nutrient cycling microbial ecosystems. These microcosms contain populations of sulphate reducing bacteria, which generate sulphide using sulphate or elemental sulphur as a terminal electron acceptor and hydrogen or organic compounds as an electron donor. This sulphide can then be oxidized into a variety of oxidation states, by diverse microbial groups, or can react abiotically with iron compounds or oxygen. Our microcosms have the advantage of retaining many of the features of the real ecosystem (such as microbial diversity, spatial structure, and abiotic interactions) while allowing the controlled manipulation of environmental perturbations.

Our microcosms develop vertical stratification of oxygen and sulphide over a period of months. We measure detailed depth profiles of sulphide and iron using a system of voltammetric microelectrodes, and oxygen depth profiles using a fluorescent oxygen probe. We also analyse the microbial community composition using barcoded 454 sequencing of the 16S rRNA gene V4 and V5 regions, as well as DGGE for the *dsrB* gene associated with dissimilatory sulphate reduction.

Using our microcosm system, we are able to reproducibly induce a sudden transition to an anoxic and sulphidic state as a function of the organic-matter loading of the ecosystem. This occurs because the organic matter contributes a greater supply of electron donors, which stimulates the growth of sulphate reducing microbes. This finding is relevant to the study of sudden regime shifts in environmental systems, such as eutrophication in lakes, or sudden

inputs of organic matter to detrital-based ecosystems – which tend to occur unpredictably. By demonstrating a controlled induction of such a regime shift, our work may improve our understanding of how these shifts happen, and can be managed, in natural ecosystems, or in important industrial processes.

Pyoverdine diversity, cross-use, and exploitation among Pseudomonas strains in natural communities

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Fluorescent *Pseudomonas* secrete the siderophore pyoverdine to scavenge iron from the local environment. While laboratory studies have shown that pyoverdine represents a public good, which can be exploited by non-pyoverdine-producing mutants (cheats), survey studies on clinical and environmental isolates have reported an enormous variety of different pyoverdine types. Here, we predict that there is a causal link between these two findings. Specifically, we argue that the presence of exploitative cheats selects for changes in pyoverdine molecule structure and receptor affinity in the producer strain, in order to make pyoverdine more exclusive to producers and less accessible to cheats. This in turn should select for *de novo* cheats in the novel pyoverdine-type background. This ongoing evolutionary antagonism is expected to drive local pyoverdine and strain diversity.

In our study, we test this hypothesis on fluorescent *Pseudomonas* strains isolated from soil and ponds. In both habitat types, we compared strains isolated from the same, close, or distant patches for their ability to produce pyoverdine, and to exploit the pyoverdine produced by other strains. If our hypothesis of diversifying selection holds true then we would expect that: (a) non-producing strains co-exist with producers in local patches; (b) pyoverdine diversity occurs at the patch level; and (c) there is local adaptation, in the sense that strains from the same patch are more likely to be able to use each other's pyoverdine than the ones produced by strains from distant patches. Furthermore, we predict that strain diversity and local adaptation should be more pronounced in soil than in water habitats, because water habitats are supposedly less structured, allowing strains to mix more easily, thereby preventing local adaptation.

Fluorescence measurements revealed that pyoverdine production levels vary greatly among natural isolates from soil and water habitats. Furthermore, pyoverdine non-producers (potentially exploitative cheats) occurred in both habitat types. We also found that the level of pyoverdine production of isolates is negatively correlated with the iron content of the habitat these isolates live in. Preliminary data of pyoverdine supplementation assays demonstrate that some non-producers are able to use pyoverdine of phylogenetically closely related strains and show both local adaptation and maladaptation in relation to pyoverdine use. Currently, we are about to check for pyoverdine diversity using iso-electric focusing. Altogether, our results suggest that an evolutionary arms race between pyoverdine producers and non-producers might occur in natural habitats, potentially driving pyoverdine diversification.

Isolation and characterization of marine Actinobacteria from Central and Southern Chile

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Due to the increase of pathogenic microorganisms resistant to antibiotics, it is priority to develop studies focused on discovering bacterial from novel ecosystems metabolites that present bioactive properties. A great number of these bioactive compounds are produced by non-ribosomal peptide synthetases (NRPS), poliketide synthases (PKS), or a combination of both. These enzymatic systems are organized in genetic clusters that encode enzymes involved in the biosynthesis of natural products with diverse biological activities. Actinobacteria represents one of the most prominent groups of bacteria for the production of bioactive compounds. The phylum Actinobacteria represents one of the largest taxonomic units of the domain Bacteria. The discovery of novel isolates belonging to the phlyum Actinobacteria and hence, its bioactive compounds, has been orientated to marine ecosystems that hold a biodiversity that is largely unexplored. In this context, the isolation of Actinobacteria in the coastal region of Valparaíso and a southern Fjord in Chile was proposed. In order to isolate a diversity of Actinobacteria, marine sediments were sampled and selective isolation techniques were used. A phylogenetic analysis was performed using the 16S rRNA gene of selected isolates. The nearly complete 16S rRNA gene sequence indicated that at least 65 isolates belong to the order of Actinomycetales, many of which could potentially be new species. These isolates belong to diverse families including Dietziaceae, Micrococcinaceae, Nocardiopsaceae and Streptomycetaceae, revealing a great biodiversity of culturable marine actinobateria. A genetic analysis detected the presence of PKS/NRPS genes in many isolates, suggesting the potential of producing bioactive compounds. This study reflects the diversity of culturable Actinobacteria associated to marine environments in coastal regions of Chile and its potential to produce bioactive compounds of biotechnological interest.

Unusual metabolic potentials revealed by monitoring changes in an AOM community in response to methylated C1 compounds

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Methylotrophic compounds such as methanol, methylamine and dimethylsulfide (DMS) are important microbial metabolic intermediates, the biogeochemistry of which will improve our understanding on carbon cycling in the environments. In this study, we designed a series of experiments to monitor the responses of an anaerobic oxidation of methane (AOM) community to methylated C1 compounds, as AOM is the major control for methane emission from marine sediments to the upper ocean.

We used an AOM-SR enrichment where ANME-2a and Marine Benthic Group D (MBGD) were the only archaea identified, as the starting material. Four parallel incubations were set up with methane, methanol, DMS and methylamine as the energy source, respectively. AOM-SR activity was observed in the incubation supplemented with methane. A notable production of methane (~2% in head space) was recorded in the incubations with methanol, DMS and methylamine, and the concentrations of sulfate nearly unchanged, after ~400 days' incubations. The structure of active microbial community in each incubation was assayed by 16S rRNA pyrotag sequencing. ANME was found as the most dominant archaea in all the incubations, with its proportion >99% in the culture with methane, 61%, 78% and 84% in the incubation with DMS, methylamine and methanol, respectively. No methanogens was detected in the culture with methane, while *Methanococcoides* spp. was identified for accounting 39%, 11% and 8% of the active archaeal community when cultured with DMS, methylamine, and methanol, respectively. The proportion of MBGD changed from less than 1% in the culture with methane, to 11% and 8% with methylamine and methanol. The increases of MBGD cells with methylamine and methanol were further confirmed by FISH

and microscopic observations. In addition, abrupt changes of the active bacterial community were observed in the cultures with different C1 compounds.

Our results further demonstrated the potential of rare microbial eco-types in response to the changes of the environmental factors, which may have a major contribution to the functional flexibility of the microbial community. Notably, ANME was the most active archaea in all the incubations, suggesting that ANME was either oxidizing methane produced by the methanogens in the culture, which seemed unlikely as we supposed AOM-SR was coupled and we observed no SR activity) or ANME possessed the capability to utilize these methylated compounds, where further tests were needed. The stimulated growths of MBGD by methylamine and methanol have extended our knowledge on the metabolic potentials of this important uncultivated archaea, which is widely distributed in marine sediments worldwide.

Nitrogen fixation acquisition of *Escherichia coli* by vector particles originating from *Klebsiella pneumonia* subsp. *rhinoscleromatis*

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We have proposed a new concept of vector particles (VPs), which is defined as “broad-host-range virus-like particles” that characterised as: broad phylogenic recipient range (Archaea-Bacteria-Eukarya) accompanied with a high generalised transduction frequency up to 2.6E-3 CFU/VP; progeny VP production from the VP-mediated transductant; spontaneous budding production without plaque formation; various discrete particle size distribution; low recipient lethality of ~10% irrespective of particle UV treatment; only electron microscopy can reveal VPs.

Should VPs play an important role in adaptation and evolution, the transfer of multiple genes through VPs would be possible between different certain species and phylogenetically distant prokaryotic groups. We focused on the transfer of nitrogen fixation genes from donor *via* VPs to the final recipient. If the hypothesis were verified eligible, the VP-mediated nitrogen fixation gene transfer would be successful, giving the final recipient the ability to fix nitrogen.

An experiment was carried out using Sago2 strain that was isolated form the rhizosphere of Sago palm tree.16S rRNA gene analysis has shown that this Sago2 has a 99.93% similarity to *Klebsiella pneumonia* subsp. *rhinoscleromatis*. Sago2 cultured in LB at 30˚C showed budding spherical particle (SG-VLPs) production. The SG-VLPs were then purified by CsCl equilibrium density gradient ultracentrifugation. Selected particles (ρ^{25}= 1.3183 - 1.2722 g/cm^3; diameter = 103.8 - 164.2 nm) were infected at multiplicity of infection of 2 at 30˚C for 15 min to the recipients.*Escherichia coli* DH5α[F$^-$; *ø80d, lacZ M15 endA1 recA1 hsdR17 (rk$^-$, mk$^-$) supE44 thi-1 l$^-$gyrA96 relA1 (lacZYA-argF)U169*]; and JE6937[F$^-$; *strR*] were chosen as recipients.

Irrespective of ultra-violet-ray irradiation, SG-VLPs showed no lethal effect on both recipients. Selection of transductants was done on N$^-$ agar plates supplemented with/without 20-µg/ml arginine, and incubated anaerobically at 30°C. The strains, which could form distinctive colonies within 5 days of incubation, were regarded as the transductants.

Results showed that SG-VLPs without UV inactivation successfully transferred N_2 fixation gene to transductants on N$^-$ agar plates. As for DH5α recipient, 4 colonies (SG-DH-trans) had N_2 fixation gene transfer frequency of 2.83 ± 3.24 E-7 CFU/SG-VLP (n=3), and for JE6937 recipient, 4 colonies (SG-E-trans) had N_2 fixation gene transfer frequency of 1.84 ± 0.80 E-8

CFU/SG-VLP (n=3). To examine N_2 fixation ability of SG-VLP transductants, 7 clones of SG-DH-trans, and 4 clones of SG-E-trans were subjected to acetylene reduction assay with controls (negative: the parental recipients; positive = Sago2). Consequently, anaerobically incubated Sago2 in LB and N^-showed nitrogenase activity. All SG-DH-trans clones exhibited the nitrogenase activity anaerobically cultured in LB, however two of them did not show the nitrogenase activity in N^-. As for SG-E-trans, no clones exhibited the nitrogenase activity in LB and N^-. Hence, different genotypic variations of transductants could be generated by VPs, while this showed that the acceptances of incorporating gene sets were greatly affected by the recipient genetic constitution.

This study clearly demonstrated that SG-VLP produced by diazotrophic bacteria, Sago2, transferred genes were responsible for introducing nitrogen fixation abilities to non-diazotrophic bacteria *via* novel horizontal gene transfer scheme.

Pyrosequencing of dddP genes revealed SAR116 clade as dominant DMS-producing bacteria in oligotrophic NW Pacific Ocean

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Dimethylsulfide (DMS) has been known to be a climatically active gas released into the atmosphere from oceans. The DMS is produced mainly by bacterial enzymatic cleavage of dimethylsulfoniopropionate (DMSP) and several DMSP lyases have been identified to date. To elucidate biogeographical distribution of bacteria relevant to DMS production, in this study, the diversity of *dddP*, which is most abundant DMS-producing gene, was investigated using newly developed primers and pyrosequencing method in the northwestern Pacific Ocean. Consistently with the previous studies, Roseobacters were major *dddP*-containing bacteria in coastal area. However, the genotypes closely related with SAR116 group were found to be a predominant fraction of *dddP*-containing bacteria in surface water of oligotrophic ocean. A DMSP-enriched culture experiment of SAR116 strain, *Puniceispirillum marinum* IMCC1322, showed that the strain can produce DMS from DMSP. Considering the huge area of oligotrophic waters and wide distribution of SAR116 group in the global ocean, they may play a significant role in climatically important DMS production and should be surely encompassed in biogeochemical studies of sulfur via bacteria-mediated DMSP degradation.

RDP: data and tools for studying structure and function of microbial communities

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RDP offers aligned and annotated rRNA and important ecofunctional gene sequences with related analysis services to the research community through its two websites, RDP and FunGene. These services help researchers with the discovery and characterization of environmental microbial communities. The current RDP release 11.2 (March 2014), offers 3,024,798 aligned and annotated quality-controlled public bacterial, archaeal, and fungal rRNA sequences. RDP recently released new alignments of bacterial and archaeal 16S rRNA gene sequence alignments and a fungal 28S gene sequence alignment using the

latest Infernal 1.1 aligner with specially-tuned covariance models (CMs). As part of RDP's efforts to support the fungal research community most RDP tools, including the RDP Classifier, Hierarchy Browser, Sequence Match, Probe Match, and RDPipeline, have been updated to work with the new fungal 28S sequence collection.

The new RDPipeline expands upon our existing high-throughput tool offerings and is designed to accommodate the latest benchtop high-throughput sequencing technologies. RDPipeline integrates with researchers' existing myRDP accounts for streamlined analysis job submission and monitoring. The new RDPipeline includes both improved performance in optimizing back-end job load distribution and increased capacity for larger datasets. It also provides additional user-friendly features such as a "my jobs" page for each user to track the job status, download results, and retrieve process parameters for past analysis tasks submitted to RDPipeline. Other enhancements include optimized paired-end read assembly (Assembler). Tested on Illumina MiSeq paired-end data, this tool outperformed its peers in selectively filtering out error-containing sequence reads, and also better handles different types of paired-end overlaps. A new data validation mechanism implemented in RDPipeline provides feedback if incorrect data input is submitted before an analysis job starts running - a feature especially valuable for inexperienced users.

RDP FunGene, RDP's Functional Gene Pipeline and Repository, offers databases of many common ecofunctional genes and proteins, as well as integrated tools that allow researchers to browse these collections and use multiple filters to choose subsets for further analysis, build phylogenetic trees, test primers and probes for coverage, and download aligned sequences. Additional FunGene tools are specialized to process coding gene amplicon data. For example, RDP FrameBot produces frameshift-corrected protein and DNA sequences from raw reads while finding the most closely related protein reference sequence. These tools can help provide better insight into microbial communities by directly studying key genes involved in important ecological processes.

Co-occurring Prymnesiophyceae hosts reveal the evolutionary diversification of the cyanobacterial symbiont UCYN-A

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Unicellular cyanobacterium *Candidatus* Atelocyanobacterium thalassa (UCYN-A) has recently been found to establish a symbiotic relationship with eukaryotic cells belonging to the Class Prymnesiophyceae in marine environment. This relationship provides clear advantages to both partners; the prymnesiophyte obtains fixed-nitrogen from UCYN-A and, in exchange, the counterpart receives fixed-carbon since UCYN-A has lost the capacity to fix carbon as well as other essential metabolic pathways. Recent studies have suggested that the same mutualistic association involving UCYN-A could be encountered in other prymnesiophytes or, even, in other taxonomic classes. However, little is known about neither UCYN-A genomic diversity nor the existence of different UCYN-A ecotypes adapted to different hosts. The aim of this study is to understand whether diverse prymnesiophyte hosts have "island-like effects" on the evolution of UCYN-A, and therefore conducting them to a host-dependent process of isolation and evolutionary divergence. First, and based on prokaryotic metagenomic analyses of 139 marine samples from the TARA Oceans expedition, we could reconstruct a 90% of the UCYN-A genome in two TARA Oceans stations from surface waters of the South Atlantic Ocean. Secondly, we applied a double

CAtalyzed Reporter Deposition Fluorescence In Situ Hybridization (CARD-FISH) to verify its presence and quantify abundance. We combined two probes targeting different prymnesiophytes with one specific UCYN-A probe. We also performed Fragment Recruitment Analyses (FRA) using the metagenomic dataset from the same location that included different size-fractions, to recruit both metagenomic reads and assemblies closely related to UCYN-A. This size-fraction metagenome sampling strategy allowed us to identify two UCYN-A populations, one was present in the size-fraction from 0.2 to 5 µm (small fraction), and a second population in the size-fraction from 5 to 20 µm (big fraction). The first population was nearly 100% identical in nucleotide identity (NI) to the only UCYN-A genome sequenced to date. In contrast, the second population showed a high divergence, around 85% NI, to the first one. Interestingly, we have also identified by CARD-FISH two different size-classes of prymnesiophyte cells associated with UCYN-A. This association was in good agreement with the size range where the two different populations of UCYN-A were found. We also detected that both the number and the position of the UCYN-A cells carried by the host was different depending on their size, i.e., small prymnesiophytes harbor 1 or 2 UCYN-A cells, and larger ones carried a kind of “symbiosome” where several (about 10) UCYN-A cells were enclosed. In conclusion, our results demonstrate that different prymnesiophyte phylotypes act as “islands” for UCYN-A diversification and are a good example of co-evolution of symbiont and host. Further single cell genome analyses on different UCYN-A populations would allow a deeper exploration of this symbiosis model.

Towards the role of mobile elements in the adaptation to life in mangrove soils

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Horizontal gene transfer (HGT) is thought to play an important role in the evolution of species and innovation of genomes, enabling acquisition of new genes or set of genes that can accelerate adaptation to new environments or changing environmental conditions. Mangroves biome presents peculiarities as fluctuations in salt concentrations and anaerobe zone occurrence, allowing the emergence of very adapted and specific community. The present study examined, by metagenomics and metatranscriptomics, the occurrence of HGT is these soils, linking such process to adaptation of microbial communities in four mangroves areas, located in the State of São Paulo, with different contamination levels [BrMgv01 (low oil contamination), BrMgv02 (high oil contamination) BrMgv03 (anthropogenic contamination) and BrMgv04 (pristine mangrove)]. The analysis was initially based on MG-RAST automated annotation, where sequences related to phages and prophages, plasmids, transposable elements, pathogenic island and gene transfer agents were detected and quantified. In all areas, the prevalence of mobile genetic elements (MGE) (encompassing sequences retrieved from transposable elements and plasmids) was observed in both approaches, based on DNA and RNA. A correlation was observed between the amount of targeted sequences and the stage of mangrove preservation; BrMgv02 showed higher numbers of RNA sequences affiliated with phages and prophages than the other samples. BrMgv04 present the most diverse mechanisms of HGT, including all classes of MGE. It might elect mangroves as system where the role of HGT has to be investigated, possibly linking the functioning of these elements with events of environmental contamination

Ecological and evolutionary perspectives on microbial carriage in the upper respiratory tract

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Bacterial carriage in the respiratory tract is a precursor to meningitis, sepsis and respiratory infection. Pneumococcal conjugate vaccines, *Haemophilus influenzae* type b and *Neisseria meningitidis* serogroup C vaccines have reduced disease cases but also profoundly modified carriage. We aimed to assess the interactions of bacteria in the upper respiratory tract via a large community-based swabbing study.

4,834 swabs from 2,417 individuals were collected during two seasons, summer 2012 and winter 2013. The swabs were assessed for the presence of *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *H. influenzae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *N. meningitidis*. The effects of demographic variables and species interactions on microbial carriage were evaluated using multi-variable binary logistic regression. Diversity of bacterial types was measured using the Simpson's Index of Diversity (1-D). Novel ecological evaluations of our dataset were undertaken using Nestedness, a measure of species organisation within swab samples. Risk ratios were used to determine the probability of co-colonisation by two bacterial species.

Regression analysis of nose swabs showed *S. pneumoniae* carriage to be affected by recent respiratory infection (Odds Ratio [OR]=1.839, p=0.001), age (p<0.001), geographical location (p=0.018), *S. aureus* (OR=0.397, p=0.004) and *H. influenzae* (OR=3.762, p<0.001). *M. catarrhalis* carriage was affected by age (p<0.001), season (OR=3.639, p<0.001) and *S. pneumoniae* (OR=1.786, p=0.037). *S. aureus* carriage was affected by age (p<0.001), season (OR=0.771, p=0.004) and *M. catarrhalis* (OR=0.305, p=0.012). *H. influenzae* carriage was affected by age (p<0.001), season (OR=2.089, p=0.006) and *S. pneumoniae* (OR=3.998, p<0.001). *P. aeruginosa* had no significant co-variates and *N. meningitidis* carriage was too low for analysis.

Diversity of *S. pneumoniae* serotypes (1-D=0.93) and sequence types (ST, 1-D=0.97) was high, with non-vaccine serotypes 6C, 11A/D and 15B/C as well as ST62 and ST199 predominating. Diversity of *H. influenzae* ST (1-D=0.99) was high, with 30.3% being novel STs. Diversity of *M. catarrhalis* STs (1-D=0.98) was also high with 58.5% being novel types.

Nestedness analysis revealed more disordered microbial communities, represented by low matrix temperatures, in individuals with recent respiratory infection (Temperature [T]=36.69°) and younger participants (T=34.75°) compared with 'healthy' (T=26.04°) and adult (T=24.89°) participants. Risk analysis showed strong positive associations between *S. pneumoniae-M. catarrhalis* (OR=4.074), *S. pneumoniae-H. influenzae* (OR=15.229) and *M. catarrhalis-H. influenzae* (OR=2.567). *S. aureus* showed negative associations with *S. pneumoniae* (OR=0.259), *H. influenzae* (OR=0.223) and *M. catarrhalis* (OR=0.247).

Our data demonstrates the diversity of the respiratory tract bacterial flora. Age, season, recent respiratory infection and bacterial interactions are important determinants of bacterial carriage. A more random order of species organisation within young and recently infected participants may be caused by weakened immunity. Our data has enabled the elucidation of respiratory community dynamics and points towards the importance of considering such dynamics in future antibiotic and vaccine development and policy.

Fighting in flow: how hydrodynamics affects bacterial evolution

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Microbiology has traditionally focused upon shaking cultures, where a mutant cell that grows faster will nearly always dominate the system. In reality, however, microbes often live in porous substrates as surface attached biofilms. Here bacteria interact with fluid as it flows through a web of interconnected pore spaces. We have developed novel microfluidic devices and mathematical models to examine how these complex environments affect evolutionary competition between bacterial strains. We focus on a system of two interconnected channels and combine porous media fluid dynamics theory with biofilm modeling to understand how bacterial growth rate links to evolutionary fitness. Paradoxically, our analysis reveals that faster growing biofilms can suffer from reduced fitness: excessive growth blocks flow, which curtails nutrient acquisition and dispersal. We confirm these predictions experimentally using microfluidic devices. Our results demonstrate how accounting for the interaction between bacterial growth and their flow environment can reverse the general prediction for microbes, that growing more rapidly will result in enhanced fitness. More generally, our framework promises to help interpret microbial phenotypes across a range of complex habitats, including in industrial settings where the maintenance of diverse bacterial populations is essential.

Niche-partitioning of *Desulfobulbus* genus along the Colne Estuary, UK

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How microbial communities assemble and how microbial species interact are two important questions that have yet to be answered in microbial ecology. This is partly due to the lack of theoretical approach that would provide a framework to understand microbial ecosystems. Established ecological theories have been tested using microbial communities to close this gap particularly after the advent of molecular ecology techniques. Still, there is not a thorough understanding of the factors that affect the microbial community assembly, biogeography and ecosystem functioning.

Previous studies have shown that the anaerobic genus *Desulfobulbus* is ubiquitous in sediments and species are differentially distributed along the Colne Estuary, UK. However, it is not clear whether niche-partitioning or interspecific competition cause this separation. Therefore, this study aims at determining if niche limitation structures the distribution of this model genus along the Colne Estuary.

Replicated anaerobic slurry microcosms were set up using sediment samples from the freshwater site of the Colne Estuary under three contrasting salinity conditions. Freshwater sediment samples were mixed with freshwater, brackish or marine nutrient-amended artificial seawater and amended with propionate as a carbon and energy source for *Desulfobulbus*. End-point samples were collected after two weeks of incubation and the *Desulfobulbus* community was analysed using denaturant gradient gel electrophoresis (DGGE) of amplified fragments of the sulphate-reduction gene *dsrB*.

DGGE results indicated significant shifts in *Desulfobulbus* populations under each condition compared to the original sediment. The freshwater microcosms were more similar to the original population than the brackish and marine microcosms. Brackish and marine microcosms had very similar DGGE patterns with most of the bands distinct from those in the

original sample. These results show a strong correlation between *Desulfobulbus* genotypes and salinity which suggests that *Desulfobulbus* species are niche-limited and cannot survive under different conditions other than their natural environment. However, since the band patterns of the brackish and marine microcosms were very similar, the extent of the niche-limitation is not clear.

This study improves our understanding as to how microbes respond to changing environments and forms a basis for future studies to test ecological theories in microbial ecology.

Rates of fixation of beneficial mutations in expanding bacterial colonies

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Evolution proceeds very differently in a population which is expanding in space, for example a growing bacterial colony, due to the 'founder effect' whereby only individuals very close to the frontier can pass on their genes into the unoccupied space, greatly increasing the amount of genetic drift, and reducing the effectiveness of selection. Recent experiments on biofilms of *E. coli* growing on agar plates indicate that the shape of the colony, in particular its roughness, seems to greatly affect the rate of fixation of beneficial mutations.

I report on recent work using a fairly detailed biophysical simulation model of a bacterial colony to explore this effect. We introduce fitter mutant cells to test the rate of fixation of beneficial mutations. By changing the amount of nutrients avilable to the cells, we can change the morphology of the growing colony, and find indeed that rates of fixation are reduced if the colony is rougher. We find that the reason for this is that the roughness increases the amount of 'mixing' between lineages at the frontier, increasing the rate at which lineages go extinct.

Evolutionary history is associated to ecosystem productivity in active marine bacterial communities

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Understanding the link between diversity and function has long been a key question in ecology. As earth is experiencing loss of diversity, the question on how it will impact ecosystem functioning remains to be answered. For terrestrial macroorganisms the original view of an hump-shaped relationship is now challenged. For marine microorganisms that drive key geochemical process and represent good community models, manipulative experiments indicate a positive relationship, but not always. However, as most studies rely on artificial communities, the link between the diversity of active bacterial communities in the environment, their phylogenetic relatedness and ecosystem functioning has never been explored. Here we show that natural assemblages that contained more distantly related active bacteria were associated to higher bacterial production, and that this positive phylogenetic diversity–productivity relationship did not dependent on community diversity. Using a long-term (7 years) survey, from surface marine bacterial communities, we also found that productivity was associated to recurrent community assembly, which further

indicates that the traits of active bacteria are an important predictor of productivity. Our results demonstrate that the evolutionary history of the active fraction of the community is important for understanding marine microbial ecosystem functioning.

Phenotype sequencing uncovers mutations in candidate genes that mediate tectivirus-resistance in *Bacillus thuringiensis*

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The family Tectiviridae is a relative rare group that includes tailless phages having a membrane beneath their icosahedral protein shell, formed of approximately equal amounts of virus-encoded proteins and lipids derived from the host cell plasma membrane. The 15 kb linear dsDNA genomes have long inverted terminal repeat sequences (~100 bp) and are coiled within the lipid membrane. This family contains two groups of phages: the lytic PRD1-*like* infecting Gram-negative enterobacteria, and the temperate ones preying on Gram-positive bacteria belonging to the *Bacillus cereus* group. Phages GIL01, GIL16, Bam35, AP50 and Wip1 are the fully-sequenced representatives of the second group. GIL01 and relatives are capable to reside as temperate phages that do not integrate into the host genome upon infection and remain as an autonomous linear plasmid in the cell. Therefore, it is important to understand the selective pressures undergone by the bacteria when facing this type of phages. The aim of this work was to study the primary interaction between these tectiviruses and their host, and the phenotypes of bacterial resistances triggered by the presence of these phages.

For this purpose, *Bacillus thuringiensis* sv. *israelensis* strain GBJ002 was subjected to a selective pressure after repetitive propagation with clear plaque (CP) mutants of GIL01 and GIL16. The CP mutants showed an elevated efficiency of killing mainly because they propagate exclusively lytically. Twenty completely tectivirus-resistant bacterial mutants were isolated. These resistant bacteria showed differences in colony morphotype and displayed distinct adaptation features, such as biofilm formation, sporulation rate, swarming motility, exopolysaccharide production and some differences in metabolic profiles. These observations indicated that tectiviruses may drive life-trait changes and ecological adaptations in the *B. cereus* group members, as results of a phage-selective pressure.

To unravel the genetic changes responsible for the phage-resistant phenotype, a whole genome sequencing method was approached. Using a pooled high-throughput sequencing analysis of multiple independent mutants, potential genes causing the tectivirus-resistant phenotype in *B. thuringiensis* were identified. Several genes associated with cell-wall metabolism and turn-over, as well as cell-surface proteins, have been pinpointed. Currently these candidate genes are been studied intensely to confirm their enrolment in the bacteriophage-resistant phenotype. These results have shed new light on the cell wall component(s) that could act as receptor(s) or mediate the interaction between these phages and their hosts. This approach will also permit to gain insights into the different strategies used by bacteria to elude phage infection.

Phylogenomic Analisis of *Prevotella* species among different ecosystems

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Prevotella are Gram-negative anaerobes, accountable for the metabolic degradation of plant polysaccharides, nonetheless, they have acquired an extensive repertoire of glycoside hydrolases that are targeted towards non-cellulosic polysaccharides. Their importance as part of the gut microbiome across phylogenies (mainly in foregut fermenters) is therefore evident; as they're regarded as good sources of cellulases for industrial biofuel processes. Besides their comensalistic roles, they are also considered opportunistic pathogens, as they can cause life threatening infections if they should escape the human colon.

We hypothesize that *Prevotella* clusters from an array of different habitats may have co-evolved with their host organs leading to a complex phylogeny according to the habitat source, and thus present a diverse array of glycoside hydrolases whose abundance changes in different habitats.

An extensive 16SrDNA sequence analysis was conducted, by using 4,608 sequences from 243 habitats directly from the public databases. Sequences were characterized based on their original isolation source, and a series of metadata categories according to the submission information. After performing a sequence alignment, the QIIME package was used to perform alpha and betadiversity analyses in order to understand the relationship between the different *Prevotella* sequences. Phylogenetic analysis was performed using FastTree with bootstraps for robustness. Analysis of *Prevotella* OTU network interactions was performed using Cytoscape. Additional comparative genomic analyses were done in IMG.

The 4,608 sequences from 243 habitats were binned into 1,272 OTUs, with 91% of the sequences classified as genus *Prevotella*, 8.9% as Paraprevotellaceae groups CF231, *Paraprevotella* or YRC22, in lower abundance. The majority of the sequences originated from gut material (hindgut and foregut sites), whereas others were from environmental and human related sites (skin, sputum, oral, vaginal sites). Phylogenetic and betadiversity analysis reveals that Prevotella species formed clades according to the host origin, most 16S rDNA sequences from foregut samples clustered together (hoatzin, bos, hyrax, sheep or kangaroo) while human gut samples clustered among those of elephants, pigs or mice. Interestingly, Prevotellas from humans were closely related to those from baboons and monkeys, denoting a primate cluster. Community interaction analysis also indicates that *Prevotella* tend to form cluster according to their host and environment. Preliminary comparative genomics shows a differential relative abundance in the Glycoside-hydrolase genes in isolates from different origins.

Our preliminary results indicate that *Prevotella* sequences are a diverse group which are influenced by the host habitat, given that they tend to form sister clades with others from related host species, and confirms that this genus is a good sources of cellulases for industrial bioalcohol production.

Mutualistic interactions maintain diversity in expanding microbial communities

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Range expansions occur at some point in the history of every microbial population. In the absence of interactions, range expansion is thought to reduce local population diversity via neutral drift. We hypothesize that mutualistic interactions between different strains could promote the maintenance of local population diversity in expanding populations.

To test our hypothesis, we engineered a model system consisting of two strains of *Pseudomonas stutzeri* that interact by cross-feeding the growth substrate nitrite. One strain consumes nitrate to nitrite while the other strain consumes the excreted nitrite. The type of interaction between these two strains is manipulated by adjusting the pH. Nitrite is toxic at low pH, thus resulting in a mutualistic interaction. The consuming strain depends on the producing strain to provide its growth substrate nitrite while the producing strain depends on the consuming strain to reduce nitrite concentrations to non-toxic levels. Nitrite is non-toxic at high pH, thus resulting in a commensal interaction. The consuming strain depends on the producing strain to provide nitrite but the producing strain does not depend on the consuming strain. We inoculated mixtures of these strains together on the center of agar plates and allowed them to expand. We then measured the emerging patterns of interacting and non-interacting strains with confocal laser scanning microscopy.

Our current results demonstrate that mutualistic interactions between the two strains maintain local population diversity in expanding populations. In the absence of an interaction, large sectors consisting of individual strains form in the expansion zone. Thus, a large proportion of the initial local population diversity was lost during expansion out of the inoculation zone. However, when a mutualistic interaction was imposed, more numerous and branched sectors formed. Thus, relatively less of the initial local population diversity was lost during expansion out of the inoculation zone. Quantitative analysis of the patterns shows that the number of sectors that leave the inoculation zone correlates positively with the strength of the mutualism. Our finding demonstrates that mutualistic interactions lead to spatial arrangements that maximize inter-strain boundaries. This presumably facilitates the exchange of nutrients between the different strains, thus leading to more rapid expansion.

Our results show that mutualistic interactions in microbial communities maintain local population diversity by changing the spatial arrangement during expansion. Future work will investigate how these spatial arrangements influence other types of inter-strain exchange processes, such as the exchange of genetic material.

Chironomid microbiome and its interaction with *V. cholerae*

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Chironomids are the most abundant insects in freshwater habitats. They undergo a complete metamorphosis of four life stages; three of which are in the water (eggs, larvae, and pupae) and a terrestrial adult stage. Chironomids were found to be a natural reservoir for Vibrio cholerae, the causative agent of cholera. *V. cholerae* was isolated from all chironomids' four life stages. To gain a better understanding of the bacterial communities that accommodate this pathogen in the insect's habitat, we studied the chironomid egg masses and larval microbiome. Using culturable as well as cloning and 454-pyrosequencing methods, we have demonstrated that endogenous bacterial communities in chironomid egg masses and larvae are abundant and diverse. The microbial community in the egg mass was distinct from that in the larva, while there were no significant differences between the microbial communities of samples of the same life stage. The differences between the bacterial communities in the egg mass and the larva may be due to the presence of one dominant bacterial taxon (28% of the total bacterial community in the larvae) which may be an insect symbiont. A large portion of the endogenous bacterial species was closely related to species known as toxicant degraders. Chironomids are known as pollution tolerant but little is known about their resistance mechanisms towards toxic substances. Bioassays based on Koch's postulates demonstrated that chironomid microbiome play a role in protecting their host from toxic hexavalent chromium and lead. *V. cholerae*, a stable resident in chironomids is present in

low prevalence. It degrades the egg masses by secreting haemagglutinin/protease, prevents eggs from hatching and exhibits host pathogen interactions with chironomids. However, the nutrients from the degraded egg masses may support the growth of the other microbiome members that have a role in protecting the host from toxicants and consequently control V. cholerae numbers in the egg masses. Therefore, the interaction between *V. cholerae* and chironomids is probably a complicated mutualistic relationship rather than a simple host–pathogen interaction.

Optimization of urban bioaerosol sampling methods for risk assessment

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Bio-aerosol contains small biological particles such as dust, bacteria, fungi and DNA fragments. As the bioaerosol could easily travel long distance and have affinity with suspended solids in the air, the possibility of causing disease such as allergic, respiratory and infection has been suggested. For assessing risk to human and ecological health due to microbial hazards in bioaerosol in urban areas, a well-established bioaerosol sampling method is required for examining microbial community diversity, populations and viability. However, method optimization and standardization have yet to be established for sampling urban bioaerosol. In this work, we compared bioaersol sampling performance of the currently used sampling methods with high volume pumping, microbial community analysis, and optimized the sampling methods in terms of total bacterial amount, microbial diversity (T-RFLP), and viability. For these purposes, Filtering method and Impinger method (with an AGI-30impinger) were used as the current aerosol sampling methods. To optimize the choice of filter, sampling duration time, and flow rate of the aerosol sampling methods, we evaluated the copy numbers of bacterial 16S rRNA gene (qPCR), and viability in the urban bioareosol samples taken in Seoul, Korea by the aerosol sampling methods. For the viability examination, the 16S rRNA gene copies of viable bacteria were measured using Propidium Monoazied (PMA) pre-treatment prior to qPCR. For PMA treatment, 12.5 μl of PMA stock were added to sample. Then samples were incubated for 45 min to make active reaction of PMA. Following incubation, samples were exposed to a 650-W halogen light for 5 min on the ice. For Filtration method, optimal operational conditions were determined as following: (i) 0.2 μ pore-size track-etched polycarbonate filter, (ii) a flow rate of 0.70 Nm^3/min, and (iii) sampling during of 24 hours, in which the amount and quality of extracted DNA and the amount of bacteria were maximized. The maximum capacity of bacteria sampling were determined as 6.24E+0.6 total bacterial 16S rRNA gene copies/Nm^3. In the case of viability results, the highest ratio of living bacterial 16S rRNA gene copies per total bacterial 16S rRNA gene copies was around 0.30 at the lowest flow rate and shortest sampling duration time tested in this work. However, the viability was significantly decreased up to 0.08 with increases in flow rate and sampling duration time. For Impinger method, the determined optimal operiontional conditions were (i) 20 ml of Peptone Broth medium, (ii) 12.5 L/min flow rate, and (iii) 30 min sampling duration time, in which the quantities of good quality DNA and total bacterial 16S rRNA gene copies were maximized. The viability results showed that Impinger method improved gaining viable bioaerosol samples. Also comparing Impinger method to Filtration method, it shows high bacterial diversity in terms of living cells. In conclusion, together with the provision of the optimal operation conditions, the findings from this work provide a useful guideline that Filtration method has advantage over Impinger method in terms of the quantities of DNA and total bacteria while Impinger method has advantage over Filtration method in terms of gaining viable bioaerosol samples.

Non-genetic Darwinian evolution of enzymes expands the substrate range of herbicide-degrading bacterial populations

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Chemostatic growth of *Delftia acidovorans* on (R)-(2,4-dichlorophenoxy-)propionate in the presence of 2,4-dichlorophenoxyacetate (2,4-D) led to the gradual adaptation of the entire population to 2,4-D as a second growth substrate. Repetitions demonstrated that this adaption occurred consistently within several weeks corresponding to over one-hundred generations. Molecular analysis revealed the absence of genetic changes indicating a non-genetic mechanism of adaptation. Accordingly, the adaptation was reliably reverted upon omission of 2,4-D. Proteomic analysis revealed random oxidative modifications (carbonylations) of the key enzyme (R)-2,4-dichlorophenoxypropionate/a-ketoglutarate-dioxygenase (RdpA), which went along with improved kinetic parameters of RdpA with 2,4-D, thereby expanding the substrate range and notably the yield of adapted individuals. As random carbonylation is known to predominantly impair enzyme activity rather than to improve it, we tested the plausibility of a chemostat-dependent selection of a subpopulation of kinetically improved population members. Mathematical modelling showed that a slightly asymmetric distribution of kinetically impaired versus improved proteins among daughter individuals together with the chemostatic elimination of slower growing individuals could explain the observed kinetic improvement of the population. We propose that in natural settings, continuously growing organisms (e.g. living in biofilms supplied by flowing water) might go through similar non-genetic adaptations providing them temporary selective advantages and increasing the chance that functionally equivalent mutations might genetically manifest the improvement.

Who's there? A global census of rumen microbial diversity

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Rumen microbes play a central role in the nutrition, health, and greenhouse gas emissions of ruminant animals. However, we do not know to what extent the rumen microbial community is the same in all ruminants, and how much host species, diet and geography influence the microbial community. The Global Rumen Census Project was established to address this knowledge gap and aims to characterise the composition and diversity of rumen microbial communities to address the following questions; 1) How much variation is there in rumen microbial communities? 2) What is the extent of diversity in each microbial group? 3) What novel groups are present? 4) Is there a core microbial community?

In total, 742 samples from a range of ruminants, and other mammals with similar digestive systems, were provided by collaborators from 58 research institutions in 33 countries. DNA was extracted from these samples, and bacterial, archaeal, protozoal, and fungal marker genes sequenced using a standardised pipeline. The dataset comprises 5 million bacterial, 1 million archaeal, 1 million protozoal, and 15,000 fungal sequencing reads.

Prevotella 1 (which includes *P*. *ruminicola* and other rumen *Prevotella* species) and the R7 group (unnamed bacteria within the order Clostridiales) were found in over 99% of samples, and overall were the most abundant bacterial taxa (mean relative abundances of 17.2 % and 10.5%, respectively). *Quinella* and *Fibrobacter* were less prevalent in samples (60.8% and 88.7%, respectively), yet were still comparatively abundant overall (1.2% and 2.7%,

respectively). *Methanobrevibacter gottschalkii* and *Methanobrevibacter ruminantium* were the most abundant archaeal taxa (46.9% and 27.1%, respectively) and were also detected in over 99% of samples. The single most abundant archaeal sequence type (following clustering at 99% similarity) represented 24.1% of all archaeal sequences, and was found in over 98% of samples, indicating that there is a high degree of similarity of methanogens in ruminants. The most abundant protozoal and fungal genera were *Entodinium* and *Caecomyces* 1, respectively.

Interrogation of sample (meta-) data will allow the identification of factors that influence which taxa are present in the rumen. The complete dataset will also serve as a valuable reference against which future rumen microbiome data can be compared.

Many of the abundant or prevalent rumen microbial taxa identified by the Global Rumen Census Project have not been adequately characterised to date, often due to a lack of available representative cultures. A related project, Hungate1000, aims to generate a catalogue of reference genomes from the rumen microbiome. Data from the Global Rumen Census are being used to inform the selection of candidates for isolation and genome sequencing.

Changes of bacterial community and chemical property of the organically enriched marine sediment under aerobic conditions

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Organically enriched marine sediment accumulated excessively in coastal area by human activities is one of the most serious problems because it affects various marine organisms. One of the possible solutions to reduce the mass of marine sediment is to promote the activity of microbial transformations, especially aerobic degradation that can make more energy for microorganisms than anaerobic degradation. However, detailed microbial processes under aerobic conditions have not yet been clear. The aim of this study was to examine changes in bacterial community and chemical components of the marine sediment under aerobic conditions by a combination of next generation sequencing and chemical analyses. Here, we focused on the marine sediment discharged by the tsunami of the Great East Japan Earthquake on March 11, 2011 in paddy fields in Higashi-matsushima, Miyagi Prefecture, Japan. The sediment was sampled on November 9, 2012, transported to the laboratory under cool conditions, and stored at 4°C until it was used. Inside of the sediment that has not been exposed to air was taken out, mixed sufficiently and then put into plastic containers under gaseous phase filled with nitrogen gas. The containers were incubated in the dark at 20-25°C for 56 days under atmospheric conditions. Over the incubation time, the sediments sampled at 0-2 mm (surface) and 12-16 mm (inside) from the surface were subjected to the measurement of pH and sulfate ion and the deep sequencing of the 16S rRNA genes to reveal changes of structure and function of the bacterial community in the sediments. Inside of the sediment, neither pH nor sulfate concentration changed, furthermore the bacterial community scarcely changed. On the other hand, at the surface of the sediment, concentration of sulfate ion increased and the pH decreased. In addition, sulfur-oxidizing bacteria occupied approximately 80% of the total bacteria after 3 days' incubation: *Sulfuricurvum* sp. of the class Epsilonproteobacteria occupied approximately 50% of the bacterial community. However, at days 7, the predominance of *Sulfuricurvum* sp. was replaced by the other sulfur oxidizing bacteria such as *Sulfurimonas* spp. and a Chromatiales bacterium which belong to the classes Epsilonproteobacteria and Gammaproteobacteria, respectively. Then, other taxonomic groups which are known as non-sulfur oxidizing bacteria

increased: at days 56, various aerobic bacteria such as Actinobacteria increased. The results in this study reveals that the reorganization of the microbial community in marine sediment placed under aerobic conditions was started by the various sulfur-oxidizing bacteria, and then other aerobic bacteria dominated.

Evaluation of the effect of a bacterial antifouling compound over extracellular polymeric substances secreted by *Nitzschia ovalis* arnott

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In the marine ecosystem there are microalgal species that secrete extracellular polymeric substances, which play a fundamental role in biofilm formation. These compounds facilitate adhesion of microalgae to surfaces, allowing the symbiotic interaction between bacteria and microalgal cells in the marine ecosystem. The formation of these biofilms is part of the first stage of the phenomenon known as biofouling.

Nitzschia ovalis arnott is a benthic diatom forming extensive biofilms and secrete extracellular polymeric substances. The EPS is composed of 95% of polysaccharides, proteins, lipids and nucleic acids. One of its main function lies in the formation of biofilms, allowing these organisms to adhere to the surface, this matrix acts as a protective barrier that provides resistance to biocides and other harmful effects; aid in the capture of nutrients and the migration of microalgae.

The marine bacteria *Alteromonas* sp. Ni1 -LEM has an inhibitory effect on adhesion of microalgae suggesting that it is a suitable candidate for the control of biofouling, however the mechanism by which this inhibition occurs is unknown.

In this work, the effect of antifouling bioactive substances over the EPS secretion of the diatom *Nitzschia* was evaluated during formation of the microalgal biofilm. For this, the concentration of total EPS secreted by the diatom co-cultivated with the bacterial compound over a 10 day period was determined. Microalgae were grown in f / 2 medium at 20 °C with constant light intensity of 100 lux. EPS concentration was determined by spectrophotometry using the phenol -sulfuric acid method. The results indicate that there are differences in the concentrations of EPS during the growth of microalgae. It was also observed a decrease in the EPS microalgal present in biofilm with the bacterial antifouling compound. Establish the possible effect of the compound with antifouling activity on the secretion of EPS of diatoms, will allow proposing a possible mechanisms to control the early stages in fouling formation.

Bacterial assembly and temporal dynamics in activated sludge of a municipal wastewater treatment plant over five years

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Activated sludge which has been the most popular biological wastewater treatment process in the past century harbors highly diverse bacterial communities and relies on them for removing various pollutants. Present knowledge of microbial communities in activated sludge mainly focuses on the bacterial diversity and abundance revealed by single grab sample or multiple samples of spatially separated WWTPs. The long-term bacterial dynamics and interactions in a WWTP remain largely unknown. In this study, we applied a correlation-

based network analysis to explore the temporal bacterial species-species and environment-species associations in activated sludge. Moreover, the temporal dynamics of bacterial community over environmental gradients (e.g., plant operational parameters and wastewater quality) were revealed and correlated to the functional stability the process.

Activated sludge sample was collected monthly from a full-scale municipal WWTP (216,000m^3/d) in Shatin, Hong Kong over five years. For each sample, DNA was extracted, PCR amplified (16S rRNA gene) and pyrosequenced. The raw sequencing data processing, including quality filtering, denoise, chimeric checking, OTU picking and classification, and diversity analysis, were conducted using QIIME. Correlation analysis and network construction were conducted using extended Local Similarity Analysis (eLSA) and Cytoscape, respectively. A python program is developed to check statistically the observed (O) and random incidences (R) of intra- and inter-taxa bacterial co-occurrence and co-exclusion correlations. The degree of the lack of agreement between O and R (O/R ratio) can be used as a benchmark for checking non-random assembly patterns in complex bacterial communities.

Utilizing large time-series 16S rRNA gene data, it is showed that in a full-scale biotechnical ecosystem like activated sludge, temporal dynamics of bacterial community shows no significant seasonal succession. Among the 15 measured variables, sludge retention time and inorganic nitrogen (NH_3-N and NO_3-N) in the aeration tank may much more significantly affect the community structure, compared with temperature, salinity, hydraulic retention time, wastewater pH, etc. Moreover, we constructed a bacterial species-species associate network consisting of 3,899 pair-wise significant correlations connecting 170 species-level OTUs and demonstrated a correlation-based statistical method to integrate bacterial association networks with their taxonomic affiliations to predict community-wide co-occurrence and co-exclusion patterns. The statistical analysis indicates that while taxonomically closely related bacteria tend to co-occur, co-excluding negative correlations are deterministically observed between taxonomically less related species, probably implicating roles of competition in shaping bacterial assembly. Overall, our results indicate that maintaining a rationally assembled microbial community structure (in terms of both diversity and abundance) is critical to sustaining long-term satisfactory and steady performance of activated sludge for pollutants removal. Realizing that the community structure is more dependent on the biological species-species interactions and can be manipulated indirectly via the control of certain key parameters (e.g., sludge retention time and inorganic nitrogen) will improve our way of operation WWTPs. Moreover, this work demonstrates that correlation-based network analysis is a powerful pool for predicting co-occurrence and co-excluding patterns in complex bacterial communities, and the identification of species-species relations will improve our understanding of ecological niches occupied by unknown species and help to predict their biological functions in ecosystems.

Effect of wood creosote on intestinal microbiota in diarrheal weaning piglets

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Weaning piglets' diarrhea is one of primary factors that cause swineherd's economic loss. Wood creosote is an anti-diarrhea medicine to have been used for about 100 years in the northeastern Asia. We investigated the change of intestinal microbiota in diarrheal weaning piglets to be fed with wood creosote.

Twelve weaning piglets (6 weeks old) were divided to 4 groups; normal, diarrheal, antibiotic fed and wood creosote fed group. The antibiotic and the creosote were supplied throughout experimental period. *Salmonella typhimurium* was inoculated to all groups except the normal group for the diarrhea induction. Fecal samples were taken the 3rd day after inoculation of *Salmonella typhimurium*. To analyze intestinal microorganisms, the pyrosequencing was performed with 16S rRNA genes of microorganisms from the fecal samples.

The normal group distributed 7 phyla including 290 species of microorganisms, the diarrheal group 12 phyla including 502 species, the antibiotic fed group 14 phyla including 505 species, and the wood creosote fed group 8 phyla including 284 species, respectively. Bacteroidetes and Firmicutes were approximately 90 percent in all groups. The ratio of Bacteroidetes was higher in the normal group than in other groups. Spirochaetes was 0.15, 5.71, 4.80, and 1.82 percent in the normal group, the diarrheal group, the antibiotic fed group and the wood creosote fed group, respectively.

We think that the supply of the wood creosote to diarrheal piglet may become similar to healthy piglet's intestinal microbiota, though this study should be progessed.

The multifaceted roles of the interspecies signaling molecule indole in *Agrobacterium tumefaciens*

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Bacteria utilize signal molecules to ensure the survival in environmental niches, and indole is an interspecies and interkingdom signaling molecule, which is widespread in the natural environment. In this study, we sought to identify novel roles of indole in soil-borne bacterium *Agrobacterium tumefaciens. A. tumefaciens* was found not to synthesize indole and to degrade it rapidly. The addition of exogenous indole dose-dependently inhibited *A. tumefaciens* growth and decreased its motility. Surprisingly, indole markedly increased *A. tumefaciens* biofilm formation on polystyrene, glass, and nylon membrane surfaces and enhanced its antibiotic tolerance. Transcriptional analysis showed that indole markedly up-regulated several biofilm-related (*celA, cheA, exoR, phoB, flgE, fliR*, and *motA*) and stress-related genes (*clpB, dnaK, gsp, gyrB, marR*, and *soxR*) in *A. tumefaciens*, which partially explained the increased biofilm formation and antibiotic tolerance. In contrast, the plant auxin indole-3-acetic acid did not affect biofilm formation, antibiotic tolerance or gene expression. Interestingly, indole was found to exhibit several similarities with antibiotics, as it inhibited the growth of non-indole producing bacteria, whereas these bacteria countered its effects by rapidly degrading indole, and by enhancing biofilm formation and antibiotic tolerance.

Metal stress alters a bacterial community's permissiveness towards plasmids

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Conjugal plasmids encoding antibiotic resistance have developed and been selected for during the antibiotic era and contribute to a growing pool of xenogenetic pollutants in the environment. When a plasmid enters a bacterial community, the community's

permissiveness, describing the exact fraction of community members that can receive a given plasmid is the key parameter to assess its spread. Transfer and maintenance of plasmids within the community are determined by specific genetic traits of plasmid, donor and recipient strains. Apart from these genetic determinants, the occurrence of stressors may modulate the acute permissiveness of a bacterial community, since plasmid transfer is considered important for stress response and adaptation to environmental change.

We hypothesized that metal stress alters the permissiveness of a complex soil bacterial community towards broad host range plasmids. Specifically, we tested if possible effects on plasmid transfer were metal specific or associated with a general stress response. We therefore had to standardize the toxicity imposed by different metals.

Metal concentrations were initially selected for five metals (Cu, Zn, Ni, Cd, As) to inhibit soil bacterial growth rates ([3H]leucine incorporation) by 25 and 50%, respectively. Further, a mCherry-tagged red fluorescent E. coli donor strain carrying the gfp-tagged broad host range plasmid pKJK5 was mixed with a soil bacterial community and exposed to the metal stressor in a filter mating assay mimicking natural nutrient conditions, with maximized cell-to-cell contact. Plasmid transfer was observed and quantified by detecting green fluorescent transconjugant microcolonies using confocal laser scanning microscopy. Transconjugants were isolated using fluorescent activated cell sorting for bacterial size, gfp-based green fluorescence and exclusion of red fluorescence. Sorted transconjugants were subsequently analyzed by 16S rRNA gene amplicon pyrosequencing.

Irrespective of the type and degree of metal stress imposed, all transconjugal pools retained a high diversity of transconjugants. However, results revealed an effect in soil bacterial community permissiveness towards broad-host-range plasmids when exposed to different stressors and to different levels of metal stress. No plasmid transfer was observed when leucine incorporation was inhibited (50%) by Cd , while all other combinations showed transfer events.

In conclusion, we demonstrate that metal stress changes the plasmid transfer ability within soil bacterial communities and suggest that this should be considered when applying metals to fields through agricultural practice.

Enhancement of compositional stability and resilience of oral microcosms towards acidification and *Candida* outgrowth by arginine supplementation

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Microbial conversion of dietary sugars into acids and the subsequent lowering of the pH in the oral cavity is generally associated with caries. Ammonia produced during arginine metabolism is a major pH-raising factor and associated with a healthy oral ecosystem. This study aims at using a multidisciplinary approach to obtain a comprehensive view of the changes introduced by elevated arginine levels on the composition and function of oral microcosms.

The multi-plaque ‘artificial mouth’ (MAM) biofilm model was inoculated with plaque-enriched saliva from a healthy volunteer. Defined mucin medium (DMM) was supplied to four stations (Control), while the remaining four were supplemented with 1.6% (w/v) L-arginine (76mM)

(Arginine). To mimic frequent intraoral cariogenic challenges, all stations received eight sucrose pulses (10% w/v, 6 min, 0.5 ml/min) a day, at two-hour intervals. Each day contained a ten-hour 'resting' period of medium alone. The medium pumps, sucrose pumps and in situ pH measurements were controlled by Labview software. The microcosms were grown for 29 days and sampled in time during both the 'resting' and 'post-sucrose' period. Organic acids (formate, succinate, acetate, lactate, propionate and butyrate) were measured using capillary ion electrophoresis. The ammonium concentration in the microcosms was assessed enzymatically.

Quantitative PCR was used to assess *Candida* load as a relative proportion of its ITS over total bacterial 16S rDNA, and to determine the abundance of two arginine deiminase system (ADS) genes, *arcA* and *sagP*, in the inoculum and microcosm samples. Microbial profiles of samples from day 7, 17 and 27 were obtained by amplicon sequencing of the V5-V7 hypervariable regions of the 16S rRNA gene using 454 GS-FLX+ Titanium chemistry and processed using the QIIME pipeline.

The pH-raising effect of arginine was confirmed by both, pH and ammonia data. Among the organic acids, acetate and butyrate concentrations were significantly lower in Arginine compared to the Control. Candida load increased in time in the Control, while it remained low in Arginine throughout the experiment. Supplementation with arginine resulted in enrichment of *arcA* and *sagP* genes. *Streptococcus* (38.7%) was the most abundant genus in the microcosms, followed by *Veillonella* (24.1%), *Actinomyces* (7.8%), *Peptostreptococcus* (5.5%) and *Megasphaera* (5.1%). OTUs designated *Megasphaera* and *Atopobium* were significantly more abundant in the Control compared to Arginine, while *Neisseria* was more abundant in the Arginine group. The differences between the two groups were not significant on day 7 ($p=0.0582$, $R=0.2969$), while both groups differed significantly at days 17 ($p=0.0264$, $R=0.4792$) and 27 ($p=0.0282$, $R=0.8519$). The Arginine samples clustered together regardless of the time point, compared to the highly dispersed Control samples. With time, the Bray-Curtis similarity among the Control group microcosms decreased, while it remained high in the Arginine group throughout the four week experimental period.

Arginine-supplementation contributed to resilience towards acidification and prevented outgrowth of opportunistic pathogen *Candida* yeasts. Arginine treatment enhanced stability of oral microbial communities and prevented their maturation: biofilms remained stable for four experimental weeks and were dominated by early colonizers of a healthy dental plaque.

The interaction of *Saccharomyces paradoxus* with its natural competitors on oak bark

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Saccharomyces cerevisiae is a well-studied laboratory model organism but its natural history is poorly understood, and confounded by domestication for wine-making. In nature, both *S. cerevisiae* and its wild relative *S. paradoxus*, are often found on the bark of oak trees, a habitat very different from sugar-rich substrates, like grape juice, to which they seem to be adapted. It is unclear therefore whether the association of yeast with oak trees is an artifact of biased sampling methods, or whether this is the natural habitat of wild yeast. Here we show that *S. paradoxus* can grow very well on oak bark medium that is sterilized by heat, filtration, or chemically. But its growth is almost completely suppressed when the microbes that normally inhabit the oak bark are present. We purified single colonies with distinguishable morphologies from oak samples and identified a set of twelve species of fungi and bacteria common in the oak infusion. We verified that this set was representative of the

oak microbiome by 454 sequencing non-cultured metagenomic DNA samples. We then tested how each species affected the growth of *S. paradoxus* in direct competition on both solid and liquid oak bark medium at 26°C (summer) and 5.5°C (winter) temperatures, and we identified both positive and negative interactions. We found that one *Pseudomonas* species produces a diffusible toxin and suppresses *S. paradoxus* almost as effectively as either the whole set of 12 species together, or the complete microbiome present in non-sterilized oak medium. Conversely, one of the 12 species, *Mucilaginibacter*, had the opposite effect on *S. paradoxus* at cold temperatures, helping it to grow better than it could alone. Microbes resident on oak bark have strong effects on the growth of *S. paradoxus* and show neutral, positive and negative interactions.

Functional communities rather than single species are involved in the conversion of methane to carbon dioxide in the environment: The role of cooperation in this process

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Lake Washington is a freshwater ecosystem which is characterized by a dynamic turnover of methane, an important greenhouse gas, serving both as major sources and major sinks. Aerobic methane-oxidizing bacteria are a key microbial group that oxidizes methane before it is released into the atmosphere, thereby acting as a natural filter. They are mainly found in the families Methylocystaceae and Beijerinckiaceae (Alpha-proteobacteria) as well as in the Methylococcaceae (Gamma-proteobacteria).

The application of deep sequencing and stable isotope probing revealed that there might be more organisms involved in the conversion of methane to carbon dioxide than aerobic methane-oxidizing bacteria alone. This idea was further supported by 454-pyrosequencing of enrichments from Lake Washington sediments, which showed co-occurring patterns of members within the family Methylococcaceae and non-methanotrophic methylotrophs within the family Methylophilaceae but also with other non-methane utilizing heterotrophs.

In this study we followed a bottom up approach and assembled artificial communities in a microcosm model system. We aimed to identify the role and contribution of cooperative behavior for methane consumption.

Therefore representative ecotypes of aerobic methane-oxidizing bacteria and non-methane utilizing heterotrophs isolated from LW were mixed to create simple artificial communities. We applied flow cytometry to determine real-time individual abundances in species mixtures. Methane consumption rates of aerobic methane-oxidizing bacteria in species mixture were measured on a gas chromatograph equipped with a flame ionization detector.

Preliminary results indicated dynamic behavior of aerobic methane-oxidizing bacteria strains of the genus *Methylobacter* and non-methanotrophic heterotrophs of the genus *Methylotenera*. Measurements of methane consumption indicated that rates were higher in mixed communities compared to pure culture control. However, more experimental data is necessary to verify these initial results.

As a preliminary conclusion, our results indicated that aerobic methane-oxidizing bacteria can benefit from the presence of non-methanotrophic heterotrophs suggesting that cooperative behaviour plays a role for methane consumption. However, the nature and stability of this cooperation needs to be further investigated.

Deep-sequencing analysis of bacterial community structure in soil contaminated by radioactive cesium

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After the occurrence the Fukushima nuclear accident, agricultural lands and forest were seriously contaminated because of radionuclide emission. Of the released radionuclides, radioactive cesium is particularly important because it has a long half-life and shares many chemical and physical properties with potassium, i.e., an important element to all organisms. Radioactive cesium is accumulated in surface soil where most of soil microorganisms colonizing through the years. Under situations that radionuclides stay around, it is likely that the growth of soil microorganisms are affected more or less because the radiation damages to the genomic DNA. Some microorganisms have been known to harbor an ability to accumulate radionuclides or to tolerate radiation. However, little is known about the change of microbial community structure in soil contaminated by radionuclides. The aim of this study was to assess how soil microorganisms are affected by radioactive cesium, especially focusing on the effect on the bacterial community structure. The soil samples were collected in Iitate Village and Nihonmatsu, Fukushima Prefecture, Japan. We sampled rhizospheric soil of the same type of plant, mugwort, so that a difference in the bacterial community structure due to geographical distance of sampling points is minimized. Concentrations of radioactive cesium of samples collected in Iitate Village and Nihonmatsu were 16,000-35,000 Bq/kg and 1,600 Bq/kg, respectively. Here, soil samples collected in Iitate were considered as a high-level contaminated soil and that in Nihonmatsu as a low-level contaminated soil. Collected samples were transported to the laboratory and then stored in a freezer at -80℃ until use. After DNA extraction and 16S rRNA-targeted PCR, we applied deep sequencing to compare the bacterial community structure. Class level analysis of the data indicated that the relative abundance of some taxonomic groups such as Sphingobacteria, Actinobacteria, Deinococci and Verrucomicrobiae became abundant in the high-level contaminated soils. Bacteria belonging to above four taxonomic groups, respectively, *Hymenobacter* sp., *Arthrobacter* sp., *Thermus thermophiles* and *Luteolibacter* sp. were increased especially in the high-level contaminated soils compared to the low-level contaminated soil. These results implicated that bacteria in soils contaminated by radioactive cesium were highly affected and the relative abundance of several bacterial groups were changed, that is, specific bacterial groups having some sorts of resistance against radionuclides dominated gradually under such situations. Analysis of bacterial community structure in soil containing much higher concentration of radioactive cesium than those described here is under progress.

Simple but effective microbial community enriched for crude oil degradation

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A microbial community was enriched for 10^{-51} dilution three years in a crude oil amended culture. The shift of the community and functional structures were evaluated by the 454 pyrosequencing, functional gene microarray, and metagenomic sequencing. In addition, the performance of crude oil degradation was analyzed. Enrichment dramatically altered microbial community structure and the functional genes and significantly increased the crude oil degradation efficiency. For example, after 10^{-20} dilution, the crude oil could be degraded within the first day of incubation. As for the microbial community, the pyrosequencing analysis revealed that the original crude oil bacterial community was dominated by

Anoxybacillus (76.74 %) and *Pseudomonas* (14.32 %). In the 10^0 dilution, after cultivated for 56 days, *Acinetobacter* became dominant with relative abundance of 85.0%-99.2%, followed by *Hyphomonas* which increased to 13.20%. In the 10^{-6} dilution, bacterial communities became simpler, dominated by *Acinetobacter* (74.76%-96.05% abundance), followed by *Pseudomonas* and *Oceanicaulis* which were minority. After one-year acclimation (10^{-36}-10^{-51} dilution), a very simple but phylogenetic and functional stable microbial community was obtained, in which the genus *Acinetobacter* accounted for more than 99.9% and was the absolutely dominant bacteria. Incubation time showed little effect on microbial diversity and community structure but obvious effect on the abundance of functional genes. The degradation ability of hydrocarbon for the long-term acclimation system kept being stimulated and active. In each incubation cycles, alkane degradation occurred early (6 days) and the degradation of aromatic carboxylic acid reinforced with 30-days incubation, while the degradation of aromatic carboxylic acid slowed down when incubation time was more than 30 days. Among the functional genes detected by GeoChip 4.0 microarray, 533 genes were detected common in all the samples no matter what the dilution times were made, 49.9% of which could be mapped to 88 KEGG pathways that were annotated by the data of metagenomic sequencing. Among them, a dozens of pathways were associated with the xenobiotics biodegradation, lipid metabolism, carbohydrate metabolism and glycosphingolipid biosynthesis. In summary, a simple microbial community was enriched with very high effectiveness in crude oil degradation, but might be weak in community stability.

Resource partitioning and simplified reconstruction of hypersaline microbial mats using meta–omics and co-culture approaches

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Marine hypersaline cyanobacterial mats are diverse laminated microbial assemblages thought to represent life on the early earth and represent a unique setting for community systems biology. The mats at Elkhorn Slough, CA are noteworthy because they produce significant nighttime fluxes of hydrogen gas and other potential biofuels as fermentation byproducts while cycling carbon, oxygen, sulfur, and nitrogen at millimeter scales. Past results indicated that net hydrogen production is a consequence of constitutive fermentation to acetate of photosynthate by Cyanobacteria and consumption by Desulfobacterales. This current study takes a top-down systems-level approach to understanding the partitioning of light and geochemical energy into metabolic activities using meta-genomic, -transcriptomic, and -proteomic techniques over a diel cycle, and also takes a ground-up approach using co-cultures of mat-derived isolates to understand mat functioning and geochemical cycling.

For diel studies, mat samples from Elkhorn Slough, CA, were sampled 9 times over a 24-hour diel cycle. To assess the role of sulfate reducing bacteria in hydrogen metabolism, mats were treated with molybdate in sulfate-free artificial seawater (to inhibit sulfate reduction) as well as control manipulations. Light, temperature, oxygen flux, hydrogen flux, organic acid flux, stable isotope, microelectrode, and acetylene reduction assay information were collected along with DNA (for metagenomes and 16S ITag amplicons), mRNA (for metatranscriptomes) and proteins (for LC-MS/MS). Based on ITags profiles, an isolation effort was then conducted on Elkhorn Slough mats to target dominant organisms. These were recombined in vitro to assess biohydrogen production performance. Metagenomic

scaffolds were used as a basis for interpreting metatranscriptomic and metaproteomic expression profiles over the diel cycle.

The most abundant annotated transcript responses were for enzymes involved in photosystem I/II and for kinase signaling in Cyanobacteria. KEGG-mapped transcriptomics results indicated two cyanobacterial daytime photosynthate storage pathways (starch and cellulose), as well as degradation pathways to glucose, and nitrogen assimilation of a variety of sources (N_2, NO_3^-, urea) by *Microcoleus chthonoplastes*. Molybdate inhibition resulted in higher hydrogen production, and higher *dsrAB* transcript abundance by members of the Alphaproteobacteria.

Cultivated representatives were isolated from 11 bacterial phyla representing all functional groups and constituting most of the identifiable DNA reads from the diel study, including 8 distinct filamentous Cyanobacteria. Genomic analyses are underway and simplified reconstructed mats and co-cultures have demonstrated recapitulation of nitrogen-fixation, light-driven sulfur cycling, and enhanced hydrogen production functions.

In order to better understand the role of extracellular polymeric substances (EPS) in facilitating metabolic interactions and nutrient fluxes among community members, we compared EPS from natural mat communities and a Elkhorn Slough mat-building isolate. Using isolate genome and community metagenome supported MS-proteomics to interrogate different subcellular fractions, we identified several hundred putative periplasmic, outer membrane, and extracellular proteins. The most abundant of these are predicted proteins involved in oxidative stress, and peptidases and putative carbohydrate degrading enzymes.

Our systems biology approach, using meta-omics data combined with fine-scale biogeochemistry and microbial isolation, provides a comprehensive understanding of the partitioning of resources and metabolic behavior in complex microbial systems.

Changes in bacterial community structure and functional diversity influenced by river water intrusion into alluvial aquifer groundwater

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Changes of environmental conditions on microbial habitats triggered by human activities could induce changes of microbial community structure and microbial activities associated with the conditions. Groundwater is heavily pumped and used for warming green houses in many agricultural areas of South Korea during cold seasons, which is named as “water curtain cultivation (WCC)”. In Wangjeon-ri, Nonsan-si, one of the most concentrated WCC green house areas, groundwater level prominently drops mostly in winter, by heavy extraction of groundwater for WCC green houses, with rising of nearby stream water level by runoff of the used groundwater. The groundwater pumping and runoff into the stream increases hydraulic head differences between groundwater and stream water, which induces surface water infiltration into the groundwater. Here we investigated changes in the microbial and functional communities within groundwater, affected by surface water intrusion, obtained along a distance from the nearby stream toward the green house dense area, including a WCC-unaffected spot. Together with analyses of geochemical and hydrologic data, the composition and diversity of microbial communities and specific functional group involved in key pathways in the geochemical cycling of Fe and sulfur were characterized using cultivation-independent analysis of both 16S rRNA and functional (dsrAB) genes. Here we

found that bacterial communities and genes encoding dissimilatory sulfite reductase alpha and beta subunits in groundwater varied along the distance from the stream, likely reflecting surface water mixing in groundwater, supported by geochemical data. Development of aerobic and/or microaerobic environments from mostly anaerobic condition of the groundwater induced changes and/or shifts of microbial communities and also associated biogeochemical activities.

Spatial structure and intra-specific competition for iron maintains bacterial biodiversity

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In natural environments we typically find a stunning diversity of bacterial species. Explaining such diversity is challenging because bacteria compete with each other for space and resources, and consequently, one would expect a few dominant species to overtake the community. Here, our hypothesis is that spatial structure and increased competition within species can stabilize co-existence between species.

To test our hypothesis, we use *Pseudomonas aeruginosa* and *Burkholderia cenocepacia*, two naturally co-occurring soil bacteria that can also be found as opportunistic pathogens of humans. It is still unclear how these two species can coexist in nature, as in laboratory experiments *B. cenocepacia* is completely eradicated by *P. aeruginosa* under well-shaken conditions. We set up experiments to test whether spatial structure and competition between different *P. aeruginosa* strains diminishes its dominance over *B. cenocepacia* and possibly enables stable co-existence. We manipulated spatial structure by growing the bacterial cultures in liquid shaken, liquid static and in viscous static medium. Due to a structured environment competing species will be clearly separated from one another, thus attenuating competitive interactions. Furthermore, we manipulated intra-specific competition by growing cultures in iron-limited medium, where bacteria rely on the secretion of sharable siderophores to scavenge iron from the environment. Under these conditions we expect the evolution of cheating mutants that no longer produce but still benefit from the siderophores secreted by the *P. aeruginosa* wildtype. Thus, we predict that the presence of cheats will lower the competitiveness of *P. aeruginosa* relative to *B. cenocepacia*.

We performed competition assays and experimental evolution by mixing the three strains, *P. aeruginosa* and *B. cenocepacia* wild type and a *P. aeruginosa* cheating mutant, in all possible combinations. We found that in pairwise competitions, co-existence was never possible although spatial structure attenuated competitive outcomes. Conversely, when all three strains grew together, co-existence became possible especially with higher degrees of spatial structure. These findings suggest an interaction effect between the level of intra-specific competition and spatial structure in shaping long-term co-existence in multi-species communities.

Nutrient and energy controls on spatial structure and community composition in assembling phototrophic biofilms

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Biological diversity has long been hypothesized to stabilize ecosystem functional properties, though the mechanisms by which diversity imparts stability to microbial systems remain poorly understood. One potential mechanism is that interspecies interactions between organisms occupying complementary niches compose networks that buffer communities against environmental variation. Environmental perturbations might substantially alter the functions of community members and the network of interspecies interactions but result in relatively minor changes to the overall composition or functional attributes of the community. To evaluate the degree to which environmental conditions influence the community structure of an assembling phototrophic biofilm and the functional roles of its individual members, we compared the primary succession of two unicyanobacterial consortia, UCC-A and UCC-O, under variable nutrient and energy input. These consortia were derived from a microbial mat within hypersaline Hot Lake, USA. A distinct cyanobacterium serves as the primary producer in each consortium, but the consortia share a nearly-identical suite of heterotrophic species. As such, the consortia are tractable in vitro model systems of constrained species richness in which to investigate the relative contributions of environmental selection and niche complementarity to ecosystem properties.

Functional capacity was assigned to individual members by partitioning metagenome sequence into species-resolved genome bins, resulting in nearly-complete reconstructed genomes for 18 of the 20 members. In addition, we designed quantitative PCR assays targeting the *rpoC* genes of all members to quantify the abundances of each heterotrophic species. Metabolic reconstructions from the genome bins further permitted the prediction of carbon and nitrogen sources usable by each community member. These reconstructions revealed that the ability to assimilate nitrate was common to both cyanobacteria but was not shared by all heterotrophic members of the consortia. As consortia were routinely cultivated with nitrate as the sole nitrogen source, we hypothesized that amendment of nitrate-containing media with a more-reduced nitrogen source would generally favor heterotroph growth. We further hypothesized that members able to incorporate alternate nitrogen sources but not nitrate would increase in relative abundance in media amended with those sources compared with nitrate-only media. We tested these hypotheses by adding alternate nitrogen sources at the inception of biofilm formation and examining the assembling biofilms' spatial structures and heterotrophic successions over time in comparison with nitrate-only biofilms. The effect of nitrogen amendment upon biofilm structure and member abundance was consortium-dependent. Ammonium amendment increased the growth of heterotrophs compared with cyanobacteria in UCC-O, but decreased relative heterotrophic growth in UCC-A. Furthermore, the inability to reduce nitrate was a poor predictor of abundance changes post-ammonium amendment, and amendment increased the abundance of some such organisms in one consortium but decreased it in the other. These data suggest that, though the consortia share their heterotrophic membership, there are significant differences in nitrogen flow between the consortia. This points to nitrogen speciation as an important driver of biofilm structure and community composition. Future work will incorporate ^{15}N stable isotope probing and metabolomics to quantify these differences in nitrogen fluxes and metatranscriptomic and metaproteomic analysis to examine the responses of consortium members to nitrogen amendment.

Island biogeography of marine aggregates: Phenotypic and genotypic species-area curves

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Detrital-based organic aggregates, e.g., marine snow and bioflocs, have been principally studied in the context of their role in carbon and nutrient cycling; little is known about their community ecology. Previously, we experimentally tested predictions of the MacArthur-Wilson theory of island biogeography using marine aggregates and their associated bacteria. Employing as metrics the metabolic capabilities and inferred functional diversity of individual aggregates, we showed aggregate-associated bacteria to be distributed and composed according to the theory's predictions, including a non-zero level of species turnover, consistency of species richness at equilibrium, and a species–area relationship falling within the range of those known for metazoans. These results support the concept that organic aggregates are sites of favorable habitat surrounded by a less favorable matrix. The goal of the present study was to further evaluate the theory by repeating the previous experiment and supplementing the culture-based metrics with culture-independent techniques, i.e., modern sequencing approaches and quantitative PCR.

In rolling-tank experiments, individual aggregates and aggregate-free water were sampled in a geometric time series lasting 35 days. On Day 4, aggregates spanning a range of sizes were collected to determine their species–area curves using phenotypic and genotypic metrics. In the case of the former, sole-source carbon substrate utilization was measured using Biolog Ecoplates; functional diversity was defined as the number of substrates utilized. For the latter, DNA was extracted, followed by pyrosequencing of the V1-V3 hypervariable region of the bacterial 16S rRNA gene.

Phenotypic metrics were consistent with previous work; larger aggregates generally had bacterial assemblages that utilized greater number of carbon substrates than those associated with smaller aggregates. Analysis of 454 pyro-tags showed that aggregates harbored 1.2 to 2.5 times more OTUs than the surrounding water. Alphaproteobacteria dominated the aggregate-free water, while aggregates were predominantly colonized by Gammaproteobacteria. On Day 4, aggregates of different sizes all harbored the same major phyla at very similar relative abundances. In contrast to the phenotypic pattern, the number of OTUs retrieved per aggregate ranged from 435 to 637 OTUs and was unrelated to aggregate size. With respect to species-area curves, therefore, we are left with discordant results from phenotypic and genotypic measures. We are repeating the experiment to test the generality of these findings.

Production of the antibiotic tropodithietic acid (TDA) is an important factor mediating colonization and competition in *Phaeobacter inhibins*

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Marine eukaryotes provide a unique habitat for surface colonization by marine microorganisms where competition between these communities is likely to influences microbial diversity. Seaweed-associated bacteria are known to produce a range of antibiotics in defense against competition and to protect the host from further colonization. For example, *Phaeobacter inhibins*, a member of the abundant marine Roseobacter clade is known to be an effective colonizer of biotic and abiotic marine surfaces. The competitive success of *P. inhibins* is thought to be, in part, due to the production of the antibiotic tropodithietic acid

(TDA), however few studies have investigated the role of this metabolite in an ecological setting. Here we used the common marine diatom *Thalassiosira rotula* as a model to investigate the role of TDA production on the ability of *P. inhibins* to colonize eukaryotic host surfaces and its influence on the structure of the natural community of the host. Batch cultures of the axenic *T. rotula* were incubated with natural seawater collected from the North Sea and inoculated with either GFP-tagged *P. inhibins* WT or a TDA-deficient mutant (WP75) and subsequent surface colonization on *T. rotula* was monitored using epifluorescence microscopy over a period of 8 days. *P. inhibins* WT colonized the surface of *T. rotula* better than WP75 and was able to out-compete other bacteria. DGGE of PCR-amplified 16S-rRNA gene fragments revealed pronounced differences in bacterial community composition between the attached and the free-living bacteria. DGGE analysis revealed that for both attached and free-living bacterial communities of the algae cultures exposed to WP75 resembled the original attached bacterial community from the North Sea. The microbial composition of both attached and free-living bacteria of algal cultures exposed to *P. inhibins* WT and WP75 will be further analyzed using a deep sequencing approach (454 pyrosequencing). Our results indicate that TDA production by *P. inhibins* WT was important in colonizing the surface of the diatom *T. rotula* and competing with other bacteria to structure the natural community in a way that will benefit the host.

In vitro **Simulation of Micro-Allelopathy on Tomato Phylloplane**

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The phylloplane is a complex micro-ecosystem that is ecologically poorly-known as compared with the rhizoplane. Abiotic (i.e. temperature, UV exposure) and biotic (i.e. interspecific competition, plant defense mechanisms) conditions make the leaf surface a hostile place to live in, therefore shaping the structure and dynamics of the microbial communities. Studies suggest that only specific microorganisms with specific adaptations are the only ones capable of thriving on the phylloplane. Of particular interest is the likely occurrence of micro-allelopathy on the leaf surface. It is presumed that, similar to soil microbes, certain foliar bacteria out-compete other species by producing biotoxins. Evidence from empirical studies however, is lacking to substantiate this claim. Compounding this is the fact that the direct detection of microbial exo-metabolites on leaves may be unsuccessful due to their extremely low concentration.This study is an attempt to establish the possibility of micro-allelopathy on the phylloplane by studying the properties of extracellular secondary metabolites that foliar bacteria produce. Bacteria were isolated from the aerial organs of tomato plants, particularly the leaves and fruits. *Pectobacterium carotovorum* subsp. *carotovorum*, a ubiquitous phytopathogen, was chosen as the 'lawn' in the antagonism assays to test the hypothesis that micro-allelopathy may likewise be beneficial to the host plant. *In vitro* methods namely two-member culture and disc-diffusion were employed to observe antagonistic interactions. The crude metabolite mixtures were subjected to temperature and pH alteration assays and subsequently re-tested to mimic *in situ* conditions. Our screening assay revealed that five percent of the the total phylloplane bacteria (n=240) was active against *P. carotovorum* subsp. *carotovorum*. Chromatographic analysis of the crude metabolite mixtures showed that well-known antimicrobials namely alkaloids, flavonoids and phenols are their major constituents. Moreover, inhibitory-lethality assays established that the metabolites were bioactive even in low concentrations. Further, the metabolites were found to be thermostable even at sterilization temperature (121^{o}C), compatible with the leaf micro-climate. Our findings proffer evidence that antagonism is indeed one of the mechanisms for persistence in the phylloplane and that such ecological interaction may act as a 'plant probiotic', benefiting the host plant by the elimination of phytopathogens.

Temporal succession of Bacteria in a coastal system

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Progress in DNA sequencing has broaden our concept of microbial communities changes in marine environments over time and space, with the coastal environment being less investigated compared to the open sea. In this study, surface bacterioplankton communities from the anthropogenic impacted Igoumenitsa, Gulf, western Greece, were investigated on a monthly basis. Samples were collected monthly from October 2012 till September 2013, from 2 m depth (25-100 m offshore), and were analyzed with 454 pyrosequencing with the use of universal bacterial primers of the V1-V3 region. Morisita similarities exceeding 60% were observed between all sites during the study except in August. Shannon and Simpson diversity indices were similar between sites, with highest values in September and October and the lowest ones in August. The major groups detected in all samples were α-, β-Proteobacteria and Bacteroidetes with total relative abundances always exceeding 65%. Sequences clustering in α-Proteobacteria, and more specifically in the genus *Pelagibacter*, were dominant (>30%) in all samples from November to April, while γ-Proteobacteria associated sequences clustering in the genera *Psychrobacter*, *Alteromonas* and *Vibrio* dominated during late spring (May) and summer. Changes in bacterial community profiles were primarily characterized by an abrupt disappearance of SAR11 between April and May and its smoother reoccurrence during autumn. SAR11 clustering phylotypes were negatively correlated to temperature and light intensity, explaining the disappearance of these OTUs in the summer months. In August, a *Vibrio* bacterial 'bloom' was observed (>50%) in the site close to the biological treatment site. Temperature, salinity, light intensity and concentration of Chla and carotenoids occurred as important factors for the ordination of the samples after redundancy analysis (RDA). This study revealed that in an anthropogenic-impacted coastal system, distinct succession patterns occur at the phylotype level, with the major players being commonly found marine Bacteria. The dominance of SAR11 during winter months further supported the global distribution of the clade not only in the open-sea but also in a semi-enclosed gulf.

The evolutionary role of plasmid populations within microbial communities

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Plasmids are self-replicating genetic elements capable of mobilization between different hosts. Plasmids often serve as mediators of lateral gene transfer, a process considered to be a strong and sculpting evolutionary force in microbial environments. Our aim was to characterize the overall plasmid population in the environment of the bovine rumen, which houses a complex and dense microbiota that holds enormous significance for humans. We developed a procedure for the isolation of total rumen plasmid DNA, termed rumen plasmidome, and subjected it to deep sequencing using the Illumina paired-end protocol and analysis using public and custom-made bioinformatics tools. A large number of plasmidome contigs aligned with plasmids of rumen bacteria isolated from different locations and at various time points, suggesting that not only the bacterial taxa, but also their plasmids, are defined by the ecological niche. The bacterial phylum distribution of the plasmidome was different from that of the rumen bacterial taxa. Nevertheless, both shared a dominance of the phyla Firmicutes, Bacteroidetes, and Proteobacteria. Evidently, the rumen plasmidome is of a highly mosaic nature that can cross phyla. Interestingly, when we compared the functional profile of the rumen plasmidome to two plasmid databases and two recently published rumen

metagenomes, it became apparent that the rumen plasmidome codes for functions, which are enriched in the rumen ecological niche and could confer advantages to their hosts, suggesting that the functional profiles of mobile genetic elements are associated with their environment, as has been previously implied for viruses.

Ecological fitness and genome stability by means of horizontal gene transfer: the case of *Candidatus* Accumulibacter phosphatis

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Enhanced Biological Phosphorus Removal (EBPR) is a variant of the activated sludge wastewater treatment process. In wastewater treatment plants in the USA, as well as in lab-scale acetate-fed EBPR reactors, the dominant organism is a member of the Betaproteobacteria in the Rhodocyclus group, named *Candidatus* Accumulibacter phosphatis. Accumulibacter is subdivided into two main Types (I and II), each of which contains several coherent clades. With the identification of these clades within the Accumulibacter lineage, questions have arisen as to whether these clades play distinct roles in EBPR.

Preliminary data indicate that Accumulibacter clade IA has higher acetate uptake rates and higher phosphate release rates, and that clade IA can reduce nitrate while clade IIA cannot. These results suggest that Accumulibacter clades inhabit different niches in EBPR ecosystems. However, the genomic architecture that triggers ecological divergence is still unidentified for Accumulibacter, encouraging us to study major differences in gene content for both clades.

From an eco-physiological perspective, it is known that genome composition can be dramatically changed through a variety of processes including horizontal gene transfer. This is recognized as a major force for genome evolution and entails the incorporation of genetic elements transferred from another organism in an earlier generation into the genome, where they form 'genomic islands', i.e. blocks of DNA with signatures of Mobile Genetic Elements (MGE). Genomic islands, whose functions increase bacterial fitness, have almost certainly been positively selected and can be termed 'fitness islands'. Here we describe a study that asks whether such fitness islands indeed confer fitness to Accumulibacter clades performing EBPR. We predicted the existence of genomic islands using bioinformatics, analyzed their prevalence, and measured their ability to excise from the host chromosome by end-point polymerase chain reaction (PCR).

In our lab-scale bioreactors, we found dominance of Accumulibacter clade IIA over clade IA and we suggest their dynamics are controlled by the presence of an integrated prophage in clade IA, which was horizontally transferred in the past. This phage-host interaction is thought to recreate the 'kill the winner' hypothesis, and this negative density-dependent selection could probably lead to altered population dynamics between both clades. We isolated free viruses from the reactors and studied the prevalence and excision capability of Accumulibacter's phage by PCR and fluorescence microscopy.

Previous research has also revealed the presence of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) associated with Cas genes in Accumulibacter. The CRISPR-Cas system functions as a prokaryotic immune system conferring resistance to foreign genetic elements such as plasmids and phages. To investigate the role of these elements in

Accumulibacter community dynamics and evolution, we analyzed seven years of samples from our lab-scale reactors for CRISPR DNA spacer acquisition.

It is envisioned that the contribution of this work will significantly improve the current knowledge on the suite of MGE and provide first insights into how these elements affect community structure and dynamics in EBPR environments. The results obtained here will clarify population dynamics of Accumulibacter, providing a more complete understanding of an important process for environmental biotechnology.

Distribution of antibiotic resistance genes in the northern Baltic Sea sediments associated with aquaculture environments

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Aquaculture farms have been suggested a hotspot for antibiotic resistance gene (ARG) enrichment and transfer due to prophylactic and therapeutic use of antibiotics to treat fish diseases. Here we used high throughput qPCR arrays to detect and quantify 296 antibiotic resistance genes, mobile genetic elements such as transposons, and 16S rRNA genes to permit gene copy number comparisons between different samples. Three biological replicates of sediment samples were collected from two medium sized of fish farms (FIN1 and FIN2) in the northern Baltic Sea during summer in 2012. We also collected sediment samples from outside the fish farms during summers in 2008, 2009, and 2012 to observe how aquaculture affects the distribution of resistance genes and mobile genetic elements. We detected 71 genes and observed a different distribution of the genes between the samples from the farms and outside the farms. The FIN1 and FIN2 farms were separated geographically but the gene distribution between the two farms was not different, indicating a similar effect of fish farming on the ARG distribution in the sediments. Antibiotics used in aquaculture (tetracycline, sulfonamide, and trimethoprim) were present at very low concentrations in sediments below the fish farms. The ARGs associated with the three antibiotics were locally enriched together with transposons in the farm sediments. Moreover, there were correlations between tetracycline resistance genes (*tetS, tetO*), trimethoprim resistance gene (*dfrA1*) and transposon genes, suggesting an association with these genes and the mobile genetic elements. Our results indicate that fish farming affects the distribution of ARGs in the northern Baltic Sea sediments.

Genetic diversity of cellulolytic enzymes in the termite-gut protists

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The relationship between termites and cellulolytic protists in their gut is a remarkable example of symbiosis, and the gut protists are essential for the survival of termites that thrive on cellulosic matter. Recent meta-EST analysis disclosed that the diverse genes for cellulolytic enzymes were highly expressed in the protistan community of the termite gut. However, the gut symbiotic protists are extremely difficult to cultivate, and diversity of the genes in individual protist species and their evolutionaly origins have not yet been clarified. In this study, we investigated cellulase genes belonging to glycoside hydrolase family (GHF) 7 and 45 of the symbiotic protists. These genes were amplified from single protistan cells and analyzed their genetic diversity.

The cells of parabasalian symbionts showing typical morphology in the hindgut suspension of termite were isolated manually and washed extensively under a microscope equipped with a micromanipulator. A single cell or a pool of 10-30 cells were subjected to isothermal whole-genome amplification (WGA) and the amplified genome DNA was used as a template for PCR with high-fidelity DNA polymerase. High-throughput pyrosequencing was performed for the amplified genes. In some cases, PCR products were cloned and the DNA sequences were determined by the Sanger method. Sequence analysis was performed using the mothur software, and genetic diversity was estimated.

Raw pyrosequencing reads were sorted by barcodes and trimmed low quality regions. After the sequence processing, we obtained total 44,459 and 105,394 reads with average read numbers in each sample were 2,779 and 6,587 for GHF7 and GHF45 respectively. Sequences reads were grouped into phylotypes; even if 95% sequence similarity was used as a criterion, two to 120 phylotypes were observed in each single cell sample. The genetic diversity was more highly observed from *Eucomonympha* sp. than *Trichonympha* sp. in both GHF genes. Sequence data of the Sanger method supported that the diversity was not caused by errors of pyrosequencing, and a number of phylotypes derived from *Eucomonympha* sp. were likely pseudogene having deletions or nonsense mutations. The results suggest the multiplication, diversification, and pseudogenization of the GHF genes within the genome of *Eucomonympha* sp.

Moreover, genetic diversity among single cells was observed. The observed diversities of cellulase genes possibly contribute to the stability of the community and the adaptation of the protists to the gut environment.

Competitive strategies differentiate two species of marine actinomycete bacteria

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Ecological interactions among bacteria are extremely challenging to study due to methodological limitations for observing microbes in their natural settings and a poor understanding of the environmental parameters that affect microbial community composition. While models for niche determination and competition have been used to describe macroecological processes, it is unknown to what extent these principles can be applied to microbial systems. Here we show that two closely related species of the marine actinomycete genus *Salinispora* are characterized by distinct competitive strategies. Using a cross-streak assay to investigate antagonistic interactions with co-occurring members of the bacterial community, we observed a temporal difference in the onset of allelopathy in the two species. The majority of inhibition observed from *Salinispora arenicola* occurred early in the growth cycle and could be linked to antibiotic production by LC-MS analysis of organic extracts from the zone of inhibition. In contrast, inhibition from *Salinispora tropica* occurred later and appeared to be due to nutrient depletion. Growth curves further showed that *S. arenicola* has significantly slower doubling times than *S. tropica*, suggesting that *S. arenicola* uses interference competition at the expense of growth while *S. tropica* employs a strategy of exploitation competition. Finally, the allelopathic effects were highly variable among strains within each species, providing experimental support for recent bioinformatic findings of extensive plasticity in the *Salinispora* secondary metabolome. This study provides strong evidence for the ecological divergence of two co-occurring and closely related species of marine sediment inhabiting bacteria.

Selection of phylogentically similar but functionally divergent microbial populations from high diversity inocula in a sequential batch reactor system

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Production of reduced chemicals such as biofuels is one of the main objectives of current environmental biotechnology. Mixed anaerobic microbial communities are resistant to invasion, exhibit functional redundancy and metabolic flexibility making them ideal for achieving maximum product yield and minimum operational cost in biofuel synthesis. Community assembly theory could inform large-scale biofuel production, which is currently limited by a lack of understanding of metabolic fluxes and the role of the different microorganisms. For example, whether system inocula or chemical environment dominates bioreactor function is unclear.

To clarify the relative contribution of inoculate versus system chemistry, we used three different complex naturally occurring microbial communities to inoculate closed sequential batch reactor systems in which glucose was the only carbon source provided and methanogenesis was suppressed. The reactors were cycled every two days for a total of 14 days. Chemical analysis and microbial composition were measured for each cycle by high-performance liquid chromatography and 16S ribosomal RNA analysis with Illumina next generation sequencing. Although chemical profile and production differed by inoculate type, similar community members were selected for in all cases. This selection was reproducible across replicates and robust to the starting community. Our results suggest that in this

system similar taxa are predictably selected for regardless of the initial inoculate, and also that these taxa can perform different functional roles under the same conditions.

Co-selection of microbial mercury and antibiotic resistance genes in gastrointestinal track of wild and farmed fish

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Mercury bioaccumulates into higher trophic levels in aquatic food webs, especially into carnivorous fish. Fish consumption is the main source of mercury exposure for humans. Wild fish have higher mercury content when compared to farmed fish of the same size because their natural diet contains more mercury and their growth rate is slower resulting in a longer exposure time to mercury.

Exposure to heavy metals such as mercury might change the microbial population in the fish gastrointestinal track that in turn may affect the health of the fish. Wild fish intestinal microbes carry mercury resistance genes together with antibiotic resistance genes. Selection pressure for heavy metals can drive co-selection of antibiotic resistance genes that are commonly moving together within mobile genetic elements.

We assume that Baltic Sea salmon (*Salmo salar*) is exposed to heavy metals more than farmed rainbow trout (*Oncorhynchus mykiss*) due to its natural diet. Selection pressure for resistance genes comes from different sources when comparing wild and farmed fish since farmed fish are treated with antibiotics for medical purposes. The aim of this study is to compare microbial resistance gene profiles between closely related wild and farmed fish to see whether they share common core resistance gene pool and how the resistance potential may differentiate as a result of metal or antibiotic selection pressure.

Metal concentration of wild and farmed fish filets will be analyzed to evaluate the metal exposure on the fish. Microbial DNA will be extracted from the contents of fish intestines. Resistance gene profiles will be analyzed with parallel qPCR method detecting over 300 metal and antibiotic resistance genes simultaneously. Mercury-resistant microbes from the intestine of fish will be enriched with cultivation on selective plates containing $HgCl_2$. Antibiotic sensitivity of cultivated strains will be tested with commercial antibiotic sensitivity test kits to show the potential co-selection.

Co-evolution is the primary force shaping termite gut microbial communities

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Termites provide an appealing model system to explore the relative effects of co-evolution and environmental factors on symbiotic gut microbiota as, unlike most insects, their gut communities are relatively complex. Lignocellulosic biomass is digested through an obligate symbiosis with specialized gut microbiota comprising bacteria and protists in lower termites and bacteria only in higher termites. Accordingly, transmission of gut microorganisms between termites is more strictly regulated than in mammals via trophallaxis (oral transmission) or coprophagy and co-speciation with the host has been observed in selected members of the gut community. Despite the successful application of culture-independent

techniques on termite gut, very little molecular data exist for Australian termite species. Here, we surveyed the gut microbiomes of 42 Australian and 24 North American termite samples, representing 16 genera, using 16S rRNA amplicon pyrosequencing. These data represent the first gut microbial community profiles for three higher (*Tenuirostristermes, Drepanotermes, Gnathamitermes*) and two lower (*Marginitermes, Porotermes*) termite genera. Microbial community profiles were compared between termite genera, diet and geographical locations. Our molecular survey revealed that while all termite genera shared a distinctive core set of microbial phyla (Bacteroidetes, Firmicutes, Spirochaetes, Proteobacteria), each termite genus had a characteristic set of microbial populations consistent with vertical inheritance. Secondarily, relative abundance of these populations reflected dietary preferences suggesting that the hindgut community adapts on shorter timescales by changes in species evenness but not constituency. These observations suggest that co-evolution is the primary force shaping gut communities in termites.

Genome flexibility and catabolic potential of alphaproteobacteria

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The α-proteobacteria is a much sequenced class of phylum Proteobacteria. They are extraordinary versatile including phototrophs, chemolithotrophs, chemoorganotrophs and aerobic photoheterotrophs. Symbiotic association of the Rhizobiaceae family bacteria with root nodules is responsible for most of the atmospheric nitrogen fixation. Other α-proteobacteria such as Rickettsiales has adopted intracellular life style as human and animal pathogens. In hydrocarbon polluted sites alphaproteobacteria are often dominating the microbial community and the much unexplored genome capability of aromatic degradation is of great interest. The aim was to study the flexibility of these genomes and their adaptation to different ecological niches, and especially to polluted soils and waters through spread and evolution of catabolic oxygenase/dioxygenase genes in six individual orders of this class.

Published genomes (231) of the alphaproteobacteria class were selected for the study and metadata was downloaded from various biological databases (NCBI, GOLD, Patric etc) for the analysis. A phylogenetic tree based on the 231 16S rRNA sequences was constructed in MEGA5 with the neighbour joining method and tree was viewed using the Interactive Tree Of Life, ITOL. Similarly the phylogenetic analysis was also done on 37 oxygenase/dioxygenase encoding genes representing nine protein families involved in the aromatic degradation using at least two representative proteins from each gene.

The 16S rRNA phylogenetic tree revealed interesting distribution of genome size, GC content and habitat. The GC content and genome size had correlation with habitat; species isolated from the rhizosphere showed higher GC content and genome size than species from other habitats. The microbes isolated from the rhizosphere and soil possessed more catabolic genes compared to those isolated from aquatic and host associated. The relative frequency of the catabolic oxygenase genes involved in aromatic compound degradation was lower in alpha- than in betaproteobacteria, but the frequency of plasmid encoded catabolic genes was relatively higher. The order Sphingomonadales had the highest frequency of plasmid encoded oxygenases. The venn diagram of aromatic oxygenase encoding genes showed that 16 of the studied oxygenases were in common to all four studied orders. The oxygenase enzymes protocatechuate 3,4 dioxygenase (pca34), protocatechuate 4,5 dioxygenase (pca45) and 4-hydroxyphenylpyruvate dioxygenase (hppDO) were the most common ones found in alphaproteobacteria. We found that species of the same genus from different habitat had significant discrepancy in genomic features like GC content and genome size eg; Methylobacterium nodulans ORS 2060 (rhizosphere) showed higher GC content, larger

genome size as well as higher frequency of catabolic genes compared to the other sequenced species in the same genus underlining the importance of habitat in genome evolution. Species from Sphingomonadales and Rhizobiales showed presence of comparatively higher amount of catabolic genes in their genomes. Phylogenetic analysis to study evolutionary relations of peripheral and central pathway oxygenases revealed clustering of these two types, with a few exceptions. In conclusion habitat seemed to play a more vital role in evolution of catabolic versatility in alphaproteobacteria than phylogenetic origin. The GC content and genome size seemed much to be varying in alphaproteobacteria genomes based on the habitats.

Use of next generation sequencing to explore bacterial diversity and ecology in an insect gut microbiome

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Sequence data from 16S ribosomal genes are increasingly used to provide rapid and cost-effective analyses of bacterial communities without dependence on isolation and culture. The advent of 'Next Generation' sequencing (NGS) together with advanced computational and statistical tools have opened up greater opportunities to use sequence data to explore community composition and dynamics, but these approaches require validation and testing. We are evaluating the use of 16S ribosomal RNA gene sequence data from the IonTorrent platform with computational and statistical tools to generate high-resolution analysis of bacterial community composition and ecology in the gut microbiome of Diamondback moth (DBM), (*Plutella xylostella* L. (Lepidoptera: Plutellidae)).

Larvae of DBM were collected from and maintained on plants containing different levels of plant defense toxins (glucosinolates): cabbage (low glucosinolate) and broccoli (high glucosinolate). After four generations, a subset of each moth population was swapped to the alternative host plant for three further generations. NGS analysis of the V3-V4 region of the 16S ribosomal RNA subunit was used to determine the baseline composition of the gut microbiota and changes in composition and abundance of bacteria in individual insects following the change in host plant.

Several major and many rare bacterial taxa were identified in individual insects in the two moth populations. We observed significance difference in presence and abundance of various taxa between individuals and moth populations. Baseline composition data confirmed distinct bacterial communities in insects maintained on low or high glucosinolate food plants. Analysis of the gut microbiome following change in host plant showed rapid changes in bacterial community composition. Computational analysis identified putative ecological associations between bacterial taxa on different host plants and in response to changes.

The validity of using Operational Taxonomic Units (OTUs) and 16S rRNA gene sequence data, and applications of statistical and computational methods to characterise bacterial community structure, composition and ecology are discussed

Skin microbial diversity of tropical frogs from the rainforest of Panama

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Many bacterial species establish symbiotic relationships with animals and plants. In amphibians, the skin microbiota plays an important role against pathogens such as the chytrid fungus *Batrachochytrium dendrobatidis*. In Panama this fungus has spread through highland and lowland forests causing dramatic declines and extinctions of amphibian species. In this study we analyze the microbial community structure on the skins of three species of frogs from one highland (Campana) and three lowland forests (Soberania, Mamoni and Nuevo Vigia) in Panama. *Agalychnis callidryas* and *Dendrophosphus ebbracatus* are arboreal tree frogs whereas *Craugastor fitzingeri* is a terrestrial species. We swabbed the skin of 139 frogs from these forests and described the bacterial community structure through Illumina sequencing of the V4 16S rRNA gene fragment. We determined operational taxonomic units based on a threshold similarity of 97% using Qiime. In parallel, we determined *B. dendrobatidis* infection status of these frogs through the use of quantitative real time PCR. Overall, the most abundant bacterial groups across the three frog species are from the orders Pseudomonadales, Actinomycetales, Burkholderiales and Xanthomonadales. Our results show that alpha diversity of skin microbial communities is higher in the terrestrial frog, *C. fitzingeri*, than in the treefrogs *A. callidryas* and *D. ebraccatus*. Principal coordinate analyses based on Unifrac phylogenetic distances show clear patterns distinguishing *C. fitzingeri* from the treefrog species. Specifically, the terrestrial frog species shows less variation and low beta diversity values between individuals than the two arboreal species. Finally, community structure comparisons between infected and non-infected frogs show no differences between these communities. Since the fungal pathogen was prevalent in all sites, the skin microbiota along with their respective hosts might already have been selected for tolerance or resistance to the pathogen. This study is the first to describe the community structure of the skin microbiota of tropical frogs through the use of culture independent techniques. Interestingly, our results indicate differences of the microbial community structure between frog species from different habitats.

The relative importance of interspecific vs. intraspecific variation during mixed community biofilm development

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Since bacteria principally exist in the environment in intimate association with countless other species, we have adapted a 3-species biofilm system, consisting of *Pseudomonas aeruginosa* PA01, *Pseudomonas protegens* Pf-5 and *Klebsiella pneumonia* Kp-1, and have systematically applied advanced transcriptomics, proteomics, imaging and use of reporter genes to investigate multi-species biofilm development and interactions of various members in the community. Using this model, we have previously shown that the mixed species biofilm displays increased stress resistance relative to the population based biofilms and that such resistance is extended to the stress sensitive biofilm members. We have also recently demonstrated that mixed species biofilm facilitates metabolic cooperation between the community members.

The three species were tagged with different fluorescent protein markers and the development of the biofilm in flow cells was followed over a period of 10 days, either as single or multispecies biofilms. The multispecies biofilm was observed to undergo a reproducible pattern of development, including dispersal and bacteriophage production. The

single species biofilms produce morphotypic variants during biofilm development, which are not detected in planktonic cultures. The percentage of variants can constitute up to 60% of the biomass. Both PA01 and Kp-1, produce a single variant while the Pf-5 produces up to five variants. The variants universally show enhanced biofilm formation and can outcompete their parental strains when grown together. Similarly, the variants perform better in the mixed species context when they replace their parental strains. However, when the mixed biofilms are generated using one of the variants, these biofilms lose the ability their enhanced stress resistance and similarly the cross protection conferred to rest of the community as observed when only the wild-type strains are present. Surprisingly, the mixed species biofilms produce fewer variants and for Kp-1 and PA01, no variants can be detected. The production of variants was also reduced when single species biofilms were exposed to cell free supernatant from the other strains. We have demonstrated that the mixed species biofilm represses the formation of genetic variants, suggesting that intraspecies variation is selected against when the interspecies variation can generate the same or increased benefits.

Microfluidic devices to measure the bacterial chemotaxis response

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Chemotaxis is a behavior by motile bacteria to sense the environment and swim in the direction of or away from chemical compounds. Chemotaxis is the result of a cascade of events, starting with interaction of chemicals via periplasmic binding proteins or directly to methyl-accepting proteins in the bacterial cytoplasmic cell membrane. This interaction induces phosphorylation of a cytoplasmic effector protein (CheY), finally leading to the flagellum modifying its rotation direction. The change of the rotation direction of the flagellum leads to the cell moving either straight forward or tumbling. In presence of an attractant gradient, the movement of the bacterium is biased in the direction of the highest concentration of this attractant. Chemotaxis is rapid, and could thus be exploitable for developing biosensors with quick response.

PDMS chips and microfluidics present versatile tools to study biological processes and cellular behavior, notably because of the flexibility of microstructure designs. Miniaturization of the field of observation may allow a decrease of the time needed to observe the chemotaxis response.

Here we pursue the design of microfluidic chips in which a gradient of attractant can be generated which enables measurement of bacterial chemotaxis. The principle of the design is based on the creation of filters composed of channels with a height of 700 nm, which allow the diffusion of small chemical molecules but prevent the passage of the bacterial cells. The chips are composed of an inner channel, where cells are introduced, connected via a filter to two parallel side channels, in which attractant solution and buffer are flowed in order to produce a gradient. The attractant molecules diffuse between the source and the sink channel, which creates a stable gradient in the inner channel.

This chip design allows the formation of a gradient of molecules perpendicular to the inner channel where the cells are swimming and reacting. The cells detect the gradient and swim toward the highest concentration of the attractant, which leads to an accumulation of cells on one side of the channel. The chemotaxis response of fluorescent bacteria is observed by microscopy and cells distribution across the channel is determined via image analysis.

We show that chemical gradients can be produced in a microfluidic design consisting of three parallel channels and that *Escherichia coli* cells introduced in the middle channel experience

chemo-attraction toward molecules, such as ribose. Moreover, this flow-based microfluidic chips offers the possibility of continuous measurements with flow of different samples and reuse of cells.

Cryptic ecology among host generalist *Campylobacter jejuni* in domestic animals

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Homologous recombination between bacterial strains is theoretically capable of preventing the separation of daughter clusters, and producing cohesive clouds of genotypes in sequence space. However, numerous barriers to recombination are known. Barriers may be essential such as adaptive incompatibility, or ecological, which is associated with the opportunities for recombination in the natural habitat. *Campylobacter jejuni* is a gut colonizer of numerous animal speciesand a major human enteric pathogen.

We quantified gene flow among 3834 7-loucs genotypes, and >200 *C. jejuni* isolates from diverse sources to investigae the origin of homologous and non-homologous recombination across the genome. The direction and magnitude of geneflow was compared to that observed in laboratory transformation experiments.

We demonstrate that the two major generalist lineages of *C. jejuni* do not show evidence of recombination with each other in nature, despite having a high degree of host niche overlap and recombining extensively with specialist lineages. However, transformation experiments show that the generalist lineages readily recombine with one another in vitro.

This suggests ecological rather than essential barriers to recombination, caused by a cryptic niche structure within the hosts.

Metagenome analysis-assisted isolation of *Bradyrhizobium* species dominated in rice roots in a paddy field of low N environments

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It has been well known that microorganisms found within plant tissues, termed endophyte, influence plant growth. There are many reports that isolate endophytes and examine their potential use as inoculants in laboratory experiments. However, little is known how they function in natural environments. N fertilization has a profound impact on the plant growth. Bacterial communities and their functional diversity in paddy rice ecosystems were drastically fluctuated with low (LN) and standard (SN) levels of N fertilizer application (0 and 30 kg N ha^{-1}, respectively). The clone library analysis of 16S rRNA genes shows that *Bradyrhizobium* sp. (OTU AP36) and *Burkholderia* sp. (OTU BP2) were abundant exclusively in the LN roots. The metagenome analysis showed the abundance of genes for plant growth promotion (acdS gene encoding 1-aminocyclopropane-1-carboxylate deaminase and the nif (nitrogen fixation) genes) was significantly increased in the LN roots. Therefore, these bacteria are expected to play important roles in the LN roots.

According to the background, our research questions are whether *Bradyrhizobium* sp. (OTU AP36) is key player in low-N-fertilizer management and may help the rice growth in the low N environment. The aims of this study are (1) the isolation of bradyrhizobia dominated in LN roots (*Bradyrhizobium* sp. (OTU AP36)) based on the metagenome analyses, (2) the verification of identity of the obtained bacterial isolates and the metagenome data by genome comparison between them, and (3) symbiotic phenotypes such as colonization of these bacterial isolates in rice seedlings and plant-growth promotion by inoculation experiments.

For the isolation of *Bradyrhizobium* sp. (OTU AP36), we adopted an isolation strategy based on oligotrophy of bradyrhizobia. After surface sterilization of rice root systems from rice plants grown in LN field, the root systems were exposed to sterilized waters for 30 days, where the water was refreshed every day. Then, they were macerated and serially diluted with sterilized waters, and plated out in HM and 1/100 strength NA media. After we picked up approximately 70 colonies with different colony morphology, DNAs from these colonies were subjected to 16S rRNA gene sequencing. As a result, we obtained three bradyrhizobial isolates belonging to *Bradyrhizobium* sp. (OTU AP36) based on 16S rRNA gene; two isolates RP7 and WD16 from HM agar medium and one isolate RP5 from 1/100 strength NA medium. To verify the identity of these isolates with metagenomic data, we determined their draft genome sequences. The genome sequences of isolates RP7, RP5 and WD16 were strongly mapped on the metagenome data of LN root microbiomes rather than SN root microbiome, although the other published bradyrhizobial genomes (USDA110, USDA6, ORS278, BTAi1 and S58) showed lower and similar mapping profiles to both LN and SN microbiomes. This result indicates that our bradyrhizobial isolates are unique to LN microbiome. Gus-tagged strain RP7 showed colonization of root tips and growth promoting effect on rice seedlings. Three isolates RP7, RP5 and WD16 showed nif gene clusters and acetylene-reducing activity in a semi-solid culture, which was suggested by metagenome analysis.

Effects of hypoxic disturbance on dynamics of benthic microbial communities and ecosystem functioning

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In marine benthic ecosystems, very little is known about changes in microbial community composition in response to disturbances such as hypoxia. Such information is crucial as microbial communities directly affect ecosystem functions and may, by producing harmful compounds, increase the disturbance-mediated loss of other benthic species. Disturbance-induced changes in microbial communities might have a significant impact on e.g. the levels of nutrients available for phytoplankton production and thus on ecosystem eutrophication, but the importance of such changes has rarely been addressed in situ.

Our objective was to provide a microbial viewpoint and elucidate the direct and indirect effects of oxygen deficiency on ecosystem functioning of soft-sediment systems. We studied changes in microbial communities in relation to changes in benthic macrofauna, and oxygen and nutrient fluxes by artificially inducing hypoxic stress for 0, 3, 7 and 48 days in a subtidal sandy habitat in the Northern Baltic Sea. We determined the number of microbes and the composition of microbial communities using terminal restriction fragment length polymorphism, next generation sequencing and quantitative PCR of 16S rRNA gene.

Microbial richness and community composition was remarkably affected by the increasing duration of hypoxic stress. Permutation based ANOVA showed that the microbial communities differed significantly from each other in all treatments, except between 0 and 3

days of hypoxia. Interestingly, the benthic macrofauna had already started changing after 3 days of hypoxia. Increasing duration of hypoxia increased the amount of Anaerolineaceae, Clostrides and sulphate reducers. This explains the observed hydrogen sulfide formation. The hypoxia and the hydrogen sulfide, most probably produced by sulfate reducing microbes, caused the macrofauna to change and decrease. Thus the hypoxic disturbance affected the microbial communities, which further affected the environment through hydrogen sulfide production, and the combination of hypoxia and hydrogen sulfide caused changes in the benthic community assemblages.

Canonical Correlation analysis showed that changes in the microbial community also correlated to increasing effluxes of ammonium, nitrate and silica, which were observed in response to increasing hypoxic stress. The ammonium efflux may be due to mineralization of organic matter or to dissimilatory nitrate reduction to ammonium by sulphate reducing bacteria. Hypoxia thus enhances the microbe-mediated eutrophication of the water column. After 48 days of oxygen deficiency, a high number of diatom terminal restriction fragments were observed, possibly derived from broken diatoms, which might explain the observed increase in the silica efflux.

The composition of microbial communities was sensitive to hypoxic disturbance and the changes in phylogenetic and physiological traits were associated with changes in ecosystem processes. This suggests that the communities directly affected the ecosystem processes investigated. It is therefore important to take microbial communities in account when ecosystem processes are modeled.

Microbial communities of wild ruminants in an altitude gradient

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The state hunting reserve Kozorog Kamnik forms part of the natural heritage of the Republic of Slovenia and is one of the largest, most preserved, and biodiverse hunting areas (43,000 ha). Due to its altitude gradient (2200 m), it is characterized by a mixture of continental and sub-polar (alpine) climates with occasional Mediterranean influences. The area receives between 1450 mm and 2200 mm of precipitation annually, peaking in spring and autumn-winter and is largely covered by woods (>68%). Five species of wild ruminants can be found distributed in this area, depending on their natural preferences: roebuck, deer, mouflon, capricorn, and chamois. Rumen contents of all five species of wild ruminants present in Slovenian alpine setting were sampled around Mt. Kosuta, where the feeding grounds of all five species overlap. Sampling was conducted during the period of state-regulated harvesting (animal culling). Samples were freeze-dried and analyzed within the Global Rumen Census project which aims to characterise the composition and diversity of rumen microbial communities globally. In addition to the samples from five species of wild ruminants, rumen samples of two autochthonous, domesticated ruminant species (bull (cika breed) and sheep (solcavska breed)) were obtained from farmers with permission of the Veterinary Service of the Republic of Slovenia. In total, microbial community data of bacteria, archaea and protozoa of 24 samples were pre-processed using standard steps in QIIME and analyzed in mothur in order to identify differences between (i) the five species of wild ruminants and (ii) between wild and domesticated ruminants. In general, significant structure in microbial communities existed for each of the species, giving rise to significant differences in structure of microbial communities between species of wild ruminants sharing different habitats. In addition, the structure of microbial communities in wild and domesticated was also significantly different.

Comparison of the microbial profiles of infected and non-infected central venous catheters – is there a potential ecological signature of infection?

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Medical devices such as peripheral or central venous catheters are now essential to modern medicine and greatly improve patient's healthcare. However, it is estimated that as many as 15% of implanted patients suffer from complications including inflammation and blood stream infections originating from these devices at any one time. These infections are frequently due to colonization of bacteria of various origins (patient, floor, linens, dressings, gloves, handling and malpractices) onto surface of implants, followed by formation of self-structured communities called biofilms. These biofilms are characterised by a high level of tolerance to antimicrobial treatments as well as host defences and constitute a source of nosocomial infections. While contaminated peripheral catheters can be routinely replaced, the distinction between a biofilm and planktonic origin for infection is challenging. Currently, the microbial ecology related to the development of deleterious infection or biofilm formation is poorly understood. In absence of biofilm biomarkers, removal of implanted devices suspected of infection constitutes a difficult therapeutic decision, frequently only relying on bacterial identification by culture-based methods that are often ambiguous and present a selective view of bacterial dynamics on these catheters. Recent literature suggests that catheter colonisation is complex, and that devices in asymptomatic patients are also colonised. Understanding the relationship between presence of non-pathogenic microorganisms and pathogenic bacteria colonising catheters may therefore lead to the identification of a microbial ecological signature with predictive value of susceptibility to future infections.

We carried out routine diagnostic culture and parallel molecular 16S rDNA analyses on 20 infected and 20 non-infected central vascular catheters (CVCs) from two French hospitals in order to determine the community composition using microbial ecological tools.

In both hospital cohorts culture identified classical pathogens in infected CVCs whereas all non-infected CVCs were culture-negative. 16S rDNA analysis detected bacteria in both infected and non-infected samples. Whereas species richness and diversity of samples varied significantly between both hospitals (species richness $p = 0.03$, 30% shared diversity), the majority of bacteria detected were of environmental origin. Most frequently detected species in infected samples were *Staphylococcus aureus*, *S. epidermidis* and *Pseudomonas aeruginosa*. Most frequently detected species in non-infected samples were *S. hominis*, *E.coli* and a *Delftia* sp. Preliminary results suggest that infected samples have a similar species richness than non-infected samples ($p = 0.46$), but have a less even community structure than non-infected samples (slope 0.17 and 0.07 respectively).

Although bacteria colonising catheters deemed as infected and non-infected by culture-based methods display strong inter-hospital variation, our preliminary community analysis revealed a difference in community structure that could be of potential importance as a marker of infective status and predictor of susceptibility of CVCs to future infection.

The big picture: Studying large anammox biofilms

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Biofilms are complex communities. Their growth and the presence of different bacterial populations can lead to physicochemical gradients in the biofilm. Those gradients could form microhabitats, allowing different bacterial populations to live in different parts of the biofilm.

One-stage partial nitritation-anammox biofilms, growing on carriers in Moving Bed Biofilm Reactors (MBBR) for nitrogen removal in wastewater treatment, is an example of a system with a strong spatial stratification. The layers facing the bulk water have access to oxygen and ammonium allowing the establishment of ammonia oxidizing bacteria (AOB). AOB in turn produce nitrite, and together with other bacteria, they create the anoxic condition that allows the establishment of anammox bacteria in deeper biofilm layers. MBBR biofilms are often several millimeters thick. These provide opportunities to studying large-scale process in biofilms.

We have studied biofilms from one-stage partial nitritation-anammox processes in MBBR. The biofilms are fixed and embedded in OCT, which allows lengthwise or crosswise cryosections of whole “wall-to wall” biofilms to be studied. Fluorescence in Situ Hybridization (FISH) and confocal laser scanning microscope (CLSM) together with novel imaging analyses tools are used to study population composition and biofilm positions of anammox and AOB, as well as eukaryotic predators.

After analyzing 16S rRNA gene clone library sequences we reported that *Brocadia* sp. 40 strongly dominated the anammox community in an MBBR biofilm. However, when studying “wall-to wall” biofilm cryosections with CLSM-FISH we observed that one of the minor members of the anammox community was present in the biofilm layers close to the bulk water, near the AOB. Thus, spatial distinct distribution between anammox populations may be due to their eco-physiological differences, just as we have shown earlier for AOB populations.

Biofilm structure and positioning of bacteria can be formed by gradients of electron donors and acceptors, but also by other forcing factors such as predation. We are using the same approach of combining cryosections and CLSM-FISH to study relationship between protozoa and bacteria in biofilms. We have shown that eukaryotic predators of different types are present at different depths in the biofilm. Preliminary result suggests that some predators penetrate into deeper biofilm parts and show evidence of grazing of anammox bacteria. Thus, neither the formation of microcolonies nor living deep within the biofilm can fully protect bacteria from predation. Little is known about bacterial predation in anammox biofilms. We discuss the importance of predation for the function of one stage MBBR nitrogen removal in wastewater.

Studies of bacterial activity in biofilms, using molecular methods, together with CLSM-FISH analyses of cryosections are underway and will be discussed in relation to electron donor and acceptor gradients and predation pressure.

The use of cryosections, combined with CLSM-FISH allows us to study large scale spatial patterns in biofilms. Together with techniques for cell activity it is possible to finally start seeing the big picture in biofilms, such as structures formed by limiting resources and predation.

Tracing signals in the meta-ome: impacts of organic enrichment on the structure and function of sediment microbes in a field experiment

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As human influence on the environment continues to expand, more ecosystems worldwide are approaching a tipping point in their tolerance of anthropogenic stress. Microbial response to anthropogenic stress has been widely described through comparisons of community structural change. However, our understanding of anthropogenic impacts to crucial microbe-mediated functions remains limited. In a novel field experiment, we combine metagenomic and metatranscriptomic approaches to compare structural and functional responses of sediment microbes to anthropogenic stress in the form of organic enrichment. To recreate organically enriched sediments typical to modified coastal environments, garden fertilizer was mixed with control sediments representing background nutrient conditions. Both enriched and control sediments were then deployed subtidally in replicate recruitment containers in Sydney Harbour, Australia (n=18). After 3, 6 and 8 weeks in the field, total DNA and RNA of the sediment community were sequenced on the Illumina HiSeq 2500 platform. Sediment-to-water fluxes of oxygen, ammonia, nitrite/nitrate, phosphate, alongside total sediment nutrients and sulphides were also measured. Sediment community structure and function differed significantly between enriched and control sediments. SSU rRNA sequences revealed higher abundances of bacteria and viruses in the enriched sediments, while archaea, eukaryotes and unclassified sequences were lower. In particular, ammonia oxidising archaea were negatively associated with enriched conditions, suggesting a reduction in the nitrogen removing potential of eutrophied environments. Negative nitrite/nitrate fluxes measured in the enriched sediments support this observation. Significant differences in 26% of mRNA sequences revealed different modes of metabolism predominating in the two treatments, with a greater abundance of sulphur metabolising genes in the enriched sediments. In contrast, photosynthesis and nitrogen metabolising genes dominated the control sediments. These results assist our understanding of the stability, resilience and redundancy of ecologically important functions that remove excess nutrients in eutrophied systems and ultimately determine repositories of carbon, nitrogen and sulphur.

Insecticide applications to soil contribute to development of *Burkholderia* mediating insecticide resistance in pest stinkbug

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We recently demonstrated that *Burkholderia* strains which are capable of degrading an organophosphorus insecticide, fenitrothion, can establish specific and beneficial symbiosis with stinkbugs *Riptortus pedestris*; thereby, making their host insects conferring fenitrothion-resistance to the host insects. We have been shown that fenitrothion-degraders are generally present at a low abundance in natural soil, and repeated applications of fenitrothion can increase the density of degraders belonging to *Burkholderia* in several soils. Some species of stinkbugs environmentally acquire free-living *Burkholderia* present in the rhizosphere every

generation. Thus, it was hypothesized that fenitrothion applications affect the dynamics of fenitrothion-degrading *Burkholderia*, thereby controlling the transmission of symbiotic fenitrothion-degrading *Burkholderia* from the soil to the stinkbugs. In this context, our objective is to evaluate the selective effects of fenitrothion on the density and diversity of symbiotic and non-symbiotic *Burkholderia* degraders and non-degraders in soil.

The microcosms were treated with fenitrothion every 2 weeks, and density and diversity of *Burkholderia* were monitored using spread-plate technique with selective medium, ARDRA finger-printing and 16S rDNA sequencing. Representative strains were isolated. To examine whether the strains could establish symbiosis with *R. pedestris*, the insects were orally administered with the strains, reared for 4 days, and then dissected to determine the infection rates of the supplied strains. The degraders were further investigated for kinetic characteristics (*Vmax* and *Km*) of fenitrothion degradation, using oxygen consumption assays. A mathematical model, based on the *Vmax* and *Km* values of the degraders, was applied to evaluate species interaction (i.e., coexistence and competition) among the degraders in the soil.

During the incubation with five applications of pesticide, the density of the degraders increased from less than the detection limit to around 10^6 g^{-1} of soil. Fenitrothion-degraders and non-degraders were isolated and identified as belonging to the genus *Burkholderia*. The 100 degraders isolated were assigned to 8 ARDRA types. The number of dominant species among the degraders declined with its density increase by fenitrothion applications, and eventually one species predominated. The simulations using *Vmax* and *Km* values for fenitrothion metabolism by the degraders showed that this dynamics of the degraders in the soil can be explained according to the competitive exclusion principle that if species share the same limiting resource they cannot coexist indefinitely, but competitively superior will outcompete the weaker ones. The isolates that established symbiosis with *R. pedestris*, were found to belong to the *Burkholderia* symbiont clade, a phylogenetic cluster that includes symbionts commonly isolated from *R. pedestris* and its relatives. The strains that did not belong to this clade could not necessarily associate with the host. The degraders in the symbiont clade predominated during the initial phase of the development of the degrader population in the microcosm.

Therefore, only a few applications of fenitrothion allow symbiotic degraders to associate with their hosts and cause the emergence of symbiont-mediated insecticide resistance.

Systemic dissecting of interspecies interactions in complex microbial communities

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Interspecies interactions via quorum sensing (QS) signalling is critical to the structure and function of microbial communities. Understanding the dynamics of interspecies interactions and the mechanisms responsible for coordinating QS-based interactions in complex communities will therefore be important for better control of the ecosystem function and behaviour. Here, the QS behaviour of a highly complex sludge community maintained in a bioreactor over 574 days was investigated using high-resolution analytical chemistry, metagenomics, metatranscriptomics and microbiology approaches, to understand mechanistically how complex communities control QS. While the sludge community is capable of synthesizing a wide range of N-acyl-homoserine-lactone (AHL) QS signals, only short and medium-chain AHLs were present in situ and were positively correlated with

ecosystem level nitrogen metabolism. Specific AHL accumulation was regulated by preferential degradation of long-chain signals by the community. An extensive isolation study further revealed that diverse microbial species, including the Proteobacteria, Actinobacteria, Bacteriodetes, Firmicutes and the Fungi that are ubiquitous in many natural and engineered ecosystems were involved in selective signal degradation, suggesting that the competing signalling activities may be prevalent in other complex communities and may have indeed evolved for the need of community to survive in highly variable and challenging environmental conditions. Thus the ecosystem level signalling biology is an outcome of differential but communal contribution by diverse microbial populations, most of which are exclusively either signal producers or degraders.

Structuring effect of oxygenation conditions on a hydrocarbonoclastic microbial community from coastal sediments

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Coastal sediments are of special relevance, housing abundant and diverse microbial communities that drive many ecosystems processes. These environments are subjected to a variety of pollutants, with crude oil being the most typical. The damaging effects of hydrocarbons on the environment and human health coupled with new societal pressures make it an urgent priority to understand the processes determining their fate. Many studies have highlighted the key role of microorganisms in the hydrocarbon degradation. However, only a few studies have taken into account the environmental conditions that are modified daily according to tide level and macrofauna activity. The induced oscillating anoxic/oxic conditions constitute a major parameter governing the organization of microbial communities. Currently, knowledge of how anoxic/oxic oscillations may affect the microbial communities and in turn hydrocarbon degradation is scarce.

In order to characterize the effect of oscillating anoxic/oxic conditions, a hydrocarbonoclastic microbial community from intertidal sediments was exposed to anoxic/oxic oscillations and crude oil in a bioreactor experiment. Ecological responses to the oscillating conditions (15 days of incubation in anoxic conditions with two aerated periods of one-day duration on days 7 and 10) were followed and compared with either permanent oxic or anoxic conditions.

Microbial communities under oscillating conditions and permanent oxic conditions were able to degrade alkanes and polycyclic aromatic hydrocarbons but in a different manner. The oscillating conditions induced a more efficient alkane biodegradation than the permanent oxic conditions. Nevertheless, polycyclic aromatic hydrocarbons removal, especially phenanthrene, was observed earlier in permanently oxic conditions than in oscillating conditions but the two periods of aeration allowed its biodegradation. Aeration periods in the oscillating conditions also stimulated bacterial activity, revealed by the increase of expression level of the 16S rRNA gene (abundance of reverse-transcribed cDNA compared to DNA). This increase in the bacterial activity was correlated with phenanthrene biodegradation. In-depth characterization of the bacterial communities by throughput 16S rRNA gene sequencing gave the opportunity to describe the microbial structure and composition and allow a better understanding of microbial interactions. The three conditions shared approximately 32% of OTUs showing the potential of many microbes to tolerate the different

oxygenation conditions. Interestingly, some of them are more abundant in oscillating conditions following the periods of aeration than in permanent anoxic or oxic conditions. Moreover 3% of OTUs were specific to the anoxic/oxic oscillating conditions. These observations pointed out that some microorganisms were adapted and/or were able to respond to dynamic environmental conditions and this probably plays a role in hydrocarbons degradation. Thus, it is crucial to take into account the environmental fluctuations such as anoxic/oxic oscillations in order to understand the role of microorganisms in determining the fate of hydrocarbon compounds in polluted marine coastal ecosystems.

Assessment of droplet-based PCR to improve the quantitative performance of microbial community analysis by massively parallel 16S rRNA gene amplicon sequencing

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Microbial community structure and dynamics are central themes in ecology and their accurate measurements are crucial to improving our understanding of microbial community functioning and stability. In recent years, next-generation sequencing (NGS) technologies have been increasingly applied to the study of complex microbial communities, both using direct metagenome and targeted 16S rRNA gene sequencing (massively parallel 16S rRNA gene amplicon sequencing, 16S-MPAS). The latter method in particular has now become relatively standard to compare large numbers of communities across habitats, time, and/or space. Such studies are also continually being improved by post-sequencing computational algorithms that minimize the impact of sequence artifacts. Notwithstanding, accurately quantifying microbial community structure/diversity remains challenging; this is mainly due to biases that are introduced during the multi-template PCR reactions used to generate amplicon libraries for sequencing. To augment the advances at the bioinformatics level, a need hence exists for technologies that mitigate the PCR-associated biases inherent to 16S-MPAS. Therefore, in this study, we assessed the feasibility of droplet-based PCR (dPCR) to improve amplification uniformity and generate more accurate species abundance estimates. We hypothesized that by segregating single or a limited number of 16S template molecules into individual PCR reactions (that is, droplets) more uniform amplification, and hence reduced detection bias, would be achieved by eliminating/minimizing competition between templates. We further predicted that the benefit of dPCR would be most pronounced at high cycle numbers, which should allow each template to reach a comparable amplicon yield irrespective of their amplification efficiency. To evaluate the performance of this dPCR-based 16S-MPAS, we used defined mock bacterial communities and assessed detection bias using both multi-template PCR and dPCR. Droplet-based PCR was performed using a commercially available droplet generator (QX100™ Droplet Generator, Bio-Rad Laboratories), amplicon libraries sequenced using the Illumina MiSeq platform, and data analyzed using QIIME. Using both equimolar and unequimolar mock communities, we observed that dPCR significantly reduced detection bias. When dPCR was performed at a single template molecule per droplet, the detection bias was reduced by three-to-five-fold as compared to traditional multi-template PCR. In addition, as expected, we also verified that measured community structures gradually converged to the expected structures as the number of dPCR cycles increased. Furthermore, increasing dPCR cycle numbers had only a marginal effect on base-call errors. In summary, we demonstrated that dPCR-based amplification is a promising approach to improve the quantitative performance of 16S-MPAS by reducing biases inherent to traditional multi-template PCR. As droplet-generating systems are becoming more readily accessible, we expect that this approach could find widespread adaptation in studies that demand accurate microbial community structure measurements. Furthermore, by inclusion of artificial 16S rRNA gene spike-ins previously developed by our

group, dPCR-based 16S-MPAS also paves the way for accurate absolute quantification of the detected phylotypes.

An interaction approach for discovering induced antimicrobial activity in bacterial soil isolates

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Soil microorganisms are the richest source of useful natural products of human interest such as antimicrobial compounds, antitumor agents, siderophores, volatiles, enzymes etc.. Many soil-inhabiting bacteria produce these secondary metabolites during interactions to suppress microorganisms competing for the same resources. However, current screening techniques for novel secondary metabolites almost exclusively target individual bacterial species under nutrient-rich laboratory conditions without accounting for the interactions that are vital in eliciting microbial activities and altering secretion of secondary metabolites.

Several studies have indicated that certain bacterial species start or increase their antibiotic production during interactions. To obtain more insight in the frequency of interaction-mediated triggering of antibiotic production we developed and applied a high-throughput method to screen for production of antimicrobial compounds by interacting bacterial species that had been isolated from the same soil habitat. In total 146 bacteria were screened for the production of antimicrobials during one-to-one confrontations in 2798 random combinations. Growth responses of two model organisms of human pathogenic bacteria, *Escherichia coli* and *Staphylococcus aureus*, were used to indicate antimicrobial activity. Our results revealed that the production of antibiotics by interacting bacteria differed often from the respective monocultures. Both positive (induction) and negative (repression) effects of interactions on antibiotic production were observed.

We discovered many bacterial isolates that showed antimicrobial activity during interactions but not in mono-cultures. Interaction triggered antibiotic production was observed for phylogenetically different combinations of bacterial isolates like *Burkholderia* sp. with *Paenibacillus* sp. and *Janthinobacterium* sp. with *Dyella* sp. and many others.

Here we will report and discuss on the significance of bacterial interspecific interactions on antimicrobial compounds production as well as on the ability of bacteria to sense and respond to different competing organisms.

Bacterial adaptation of a methanol-fed denitrifying marine biofilm to batch mode conditions

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A methanol-fed fluidized denitrification reactor was used by the Montreal Biodome (a natural science museum) to control the nitrate levels in its three million liter seawater aquarium. The reactor was operated in continuous mode and was composed of carriers supporting the growth of a denitrifying biofilm. The bacterial biota in the biofilm was estimated to contain between 15 and 20 species, among which around 80% were affiliated to *Methylophaga nitratireducenticrescens* JAM1, involved in reduction of nitrate to nitrite only, and to *Hyphomicrobium nitrativorans* NL23, capable of full denitrification. A previous study by our group recommended that the denitrification system should be operated in batch mode to

facilitate its management. Although appropriate changes were made, the new configuration failed in delivering efficient denitrifying activities. One hypothesis is that the microbial biota of the biofilm could not adapt properly to the batch mode. Further studies were then performed to assess the behaviour of the denitrifying biofilm in batch mode.

The denitrifying biofilm developed in the continuous mode were detached from the carriers, homogenized, and inoculated in artificial seawater containing new carriers, and supplemented with nitrate and methanol. Four conditions were tested with nitrate concentrations at 300 or 900 mg-N/L, and temperatures at 23°C or 30°C. These cultures were incubated in denitrifying conditions, and each week, only the carriers with the biofilm were transferred into fresh media. After 5 passages, the biomass, the nitrate and the nitrite concentrations were measured. Our results showed that the denitrifying rates were higher at 30°C than at 23°C. We also varied the concentration of NaCl (0%, 0.5% and 1%) in the artificial seawater, with nitrate at 300 mg-N/L and temperature at 23°C. The denitrifying rates were higher in the 0% and 0.5% NaCl seawater than the ones in full-strength seawater (2.75% NaCl). The lowest denitrifying rates of all the tested conditions occurred in the 1% NaCl seawater. PCR-DGGE profiles, qPCR analysis and pyrosequencing of the 16S rRNA gene sequences were performed to assess variations in the bacterial populations. *M. nitratireducenticrescens* JAM1 increased in proportion in all conditions. A thousand-fold decrease of *H. nitrativorans* NL23 occurred in the full-strength seawater and in the 1% NaCl seawater, but its persistence in the 0 and 0.5% NaCl seawater was observed. This suggests that other microorganisms have taken over the full denitrification activities in the full-strength seawater. Sequences affiliated to *Marinicella* spp. were found to represent between 1.5 to 5% of the pyrosequencing reads in full-strength seawater. Other sequences affiliated to *Paracoccus, Pseudomonas* and *Stappia*, genera in which some species are known to be denitrifiers, were found but represented below 0.3% of the reads. These results suggest that changes in the denitrifying biofilm populations occurred to adapt to new environmental conditions.

Co-cultivation suggests that pathogenicity of *Dinoroseobacter shibae* towards *Prorocentrum minimum* is controlled by quorum sensing and CtrA-phosphorelay

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Members of the *Roseobacter* clade within the *Alphaproteobacteria* often dominate bacterial communities associated to marine algae. Studying the interactions between *Roseobacters* and marine algae may provide a better understanding of their roles in the marine biogeochemical cycles. Our recent work using the *Roseobacter* strain *Dinoroseobacter shibae* and the phototrophic dinoflagellate *Prorocentrum minimum* as an experimental model system has demonstrated that the interactions between these two organisms switch from mutualistic to pathogenic, thus resembling the “Jekyll and Hyde” interaction proposed for *Emiliania huxleyi* and *Phaeobacter inhibens*. Here we are starting to unravel the molecular mechanisms underlying this relationship.

We used RNA-seq to analyze the transcriptome of *D. shibae* in the co-culture with *P. minimum* at three different growth stages. We found that the quorum sensing (QS) genes and the genes of the CtrA-phosphorelay as well as the flagella genes are regulated at different stages, indicating their potential roles in the interactions of *D. shibae* and *P. minimum*. To test whether QS and CtrA play a role in the algal – bacterial interaction, we cultivated the QS null mutant Δ*luxI*$_1$ and the Δ*ctrA* deletion mutant together with *P. minimum* and monitored their growth dynamics using flow cytometry. The data show that the

pathogenic effect of the QS null mutant occurred earlier than that of the wild-type strain. However, the Δ*ctrA* mutant strain had completely lost the ability to kill algae. Thus, we demonstrated that QS and CtrA-phosphorelay control pathogenicity of *D. shibae* towards *P. minimum*.

Evenly distributed and unique bacterial community on the biofilm of granular active carbon PreBiofilter in bench-scale pilot plants for surface water pretreatment

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A bench-scale pilot plant of PreBiofilter was constructed to process surface water from the Canoochee River, Georgia, USA. DNA was extracted from the raw water, biofilm on PreBiofilters and filtrate water. Length Heterogeneity PCR (LH-PCR) of bacterial 16S rRNA gene followed by cluster analysis of bacterial operational taxonomic unit (OTU) was used to analyze bacterial diversity and communities. PCR products of 16S rRNA gene were further identified through cloning and sequencing. Total organic matter (TOC) and dissolve organic carbon (DOC) were determined in raw surface water and filtrates.

PreBiofilter exhibited a significant reduction of 12.6% total organic carbon (TOC) and 10.1 % dissolved organic carbon (DOC). The evenness and Shannon diversity of bacterial OTUs are significantly higher on the biofilm of PreBiofilter than in raw water and filtrates. Similar bacteria communities were observed in the raw water and filtrates using relative abundance of bacterial OTUs. However, the bacterial communities in the filtrates were relatively similar to those in the PreBiofiler using presence/absence of bacterial OTUs.

Different substrates of either GAC or raw and filtrated water greatly contribute to the abundance of bacteria; whereas, bacteria shear from colonized biofilm of PreBiofilters and enter filtrates. Evenly distributed and diverse bacteria in the biofilm of PreBiofilters may play an important role to remove organic matters from surface water thus are important for both economic and safety way for conventional surface water treatment.

A field guide to uncultivated marine bacterial and archaeal clades

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As the number of sequences from uncultivated marine Bacteria and Archaea has surpassed tens of thousands of sequences, this study investigates the usability of a standardized phylogenetic and taxonomic framework to phylogenetically organize all sequences from uncultivated organisms in the SILVA small subunit (SSU) ribosomal RNA gene database.

In order to achieve this goal, we constructed phylogenetic trees based on full-length 16S rRNA gene sequences, and applied a candidate taxonomic unit (CTU) classification system, along with a standardized nomenclature. Known marine bacterial and archaeal clades, which were already annotated on the SILVA SSU ribosomal RNA gene reference tree, were mapped to their original publications. In addition to these "published" marine clades, which are limited to 20-30 groups, text-mining procedures were performed on the SILVA SSU rRNA databases to determine additional groups exclusively (or mostly) marine. The new taxonomic

classification was complemented with a standardized nomenclature, which we believe will enhance the comparability of microbial ecological diversity studies.

Based on this work we analyzed a large-scale meta-analysis of publicly available 16S rRNA amplicon datasets (specifically the International Census of Marine Microbes dataset) to gain insights into the global distribution of various marine clades, their ecology, biogeography, and interaction with oceanographic variables.

Our results provide an updated view on the distribution of widely known marine clades; as our CTU approach breaks down these randomly lumped clades into smaller objectively calculated subgroups. Plus, with the exhaustive text-mining approach many new marine clades are now recognized and named, and can be used as a guide in future studies.

Acidobacteria – they are not only in soil – An updated phylogenetic and taxonomic framework for the phylum Acidobacteria

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Among the taxa highly recalcitrant to cultivation, the bacterial division Acidobacteria has been the focus of attention, as it is the second most abundant phylum in soils based on 16S rRNA surveys. Yet very little is known of the ecology and the role of its members in global biogeochemical cycles.

In this study we have sought to reconcile the accumulating sequences of Acidobacteria in the form of a new phylogenetic reconstruction, and to apply a novel taxonomic hierarchy on this phylogeny. As there are not many cultivated organisms within Acidobacteria, this phylum is poor in terms of formally named taxa. This novel taxonomic hierarchy is based both on the phylogenetic reconstruction and the identity of the sequences within the clades. This new taxonomic hierarchy was complemented with a standardized nomenclature, as proposed by Yarza *et al.* 2014. With this approach, we annotated 23 classes within the Acidobacteria phylum. At lower taxonomic levels, we annotated 58, 134, and 1603 taxa orders, families and genera, respectively. These numbers are a tremendous addition to the currently known three orders, four families and 11 genera of Acidobacteria.

In an effort to characterize the environments that different acidobacterial taxa originate from, we annotated each sequence in our tree with structured habitat terms deduced from their associated metadata. Our results suggest that freshwater, marine, and soils harbor different lineages of Acidobacteria, and that most Acidobacteria is found in particle-rich environments. These results were complemented with a survey of amplicon and metagenome datasets from diverse environments, and further confirmed the existence of environment specificity of certain lineages of Acidobacteria.

Feeding by the chlorarachinophyte *Bigelowiella natans* on *Synechococcus*

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We investigated feeding by the chlorarachniophyte *Bigelowiella natans* on diverse strains of the marine cyanobacterium *Synechococcus*. We also measured the ingestion and clearance rates of *B. natans* on *Synechococcus* CC9311 and CC9605 as a function of prey concentration as well as growth rates on these strains. The chlorarachniophyte *B. natans* was found to feed mixotrophically on diverse strains of *Synechococcus*. The maximum ingestion rates of *B. natans* on *Synechococcus* CC9311 and CC9605 were 2.77 and 2.18 cells predator^{-1} h^{-1}. The maximum clearance rates of *B. natans* on *Synechococcus* CC9311 and CC9605 were 197.2 and 331.5 nl predator^{-1} h^{-1}. The maximum growth rates (mixtrophic growth) of *B. natans* on *Synechococcus* CC9311 and CC9605 were 0.38 d^{-1} and 0.28 d^{-1}, while its growth rates (phototrophic growth, mean ± SD) were 0.174 ± 0.019 d^{-1}. The results of the present study suggest that *B. natans* is effective protistan grazer of *Synechococcus* in marine planktonic food webs.

Effects of different diet composition and environmental factors on Atlantic salmon (*Salmo salar* L.) gastrointestinal tract microbial community

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Diet composition, husbandry and farm environments likely influence the abundance and composition of microbial communities in the gastrointestinal tract of maricultured Atlantic salmon, which in turn may act as an indicator of fish health and growth rates. The aim of this study was to understand the potential influences of different diet compositions and other measurements made to gastrointestinal tract microbial communities of Atlantic salmon during a feeding trial in small (125 m^3) sea cages on a commercial farm.

Salmon were fed four different diet formulations: 1) a standard commercial diet with normal fishmeal level (diet 1); 2) a standard commercial diet with low fishmeal level (diet 2); 3) a high protein diet (diet 3); and 4) a high lipid diet (diet 4) over five sampling occasions over a 6 month interval. Microbial communities were analysed by 16S rRNA-based pyrosequencing with 10 fish sampled per time point per diet. Canonical analysis of principal coordinates and PERMANOVA indicated separation and significant differences among diet groups ($p=0.003$) and time of sampling ($p<0.001$). The results also suggested significant differences between bacterial groups that may be related to the diet, and the abundance of Vibrionaceae, cyanobacteria and lactic acid bacteria were distinguishable among the different of diets and the time of sampling (seasonal effect).

An interesting observation was the significant number of cyanobacterial reads especially during November, January and March. These reads included mainly filamentous or coccoidal forms of cyanobacteria. The overall data demonstrated dynamic salmon gastrointestinal microbial communities that were influenced by the different diet compositions and seasonal effects.

The evolutionary divergence of *psbA* gene in *Synechococcus* and their myoviruses in the East China Sea

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Marine *Synechococcus* is a principal component of the picophytoplankton and makes an important contribution to primary productivity in the ocean. Synechophages, infecting *Synechococcus*, are believed to have significant influences on the distribution and abundance of their hosts. Extensive previous ecological studies on cyanobacteria and viruses have been carried out in the East China Sea (ECS). Here we investigate the diversity and divergence of *Synechococcus* and their myoviruses (Synechomyoviruses) based on their shared photosynthesis *psbA* gene. *Synechococcus* is dominated by subclades 5.1A I, 5.1A II and 5.1A IV in the ECS, and clades SMI and SMII are the dominant groups in the Synechomyoviruses. As two phylogenetically independent clades, there is much higher diversity of the Synechomyoviruses than *Synechococcus*. Obvious partitioning characteristics of GC and GC3 (the GC content at the third position of each codon) are obtained among different picophytoplankton populations and their phages. The GC3 content causes the *psbA* gene in *Synechococcus* to have a higher GC content, while the opposite is true in the Synechomyoviruses. Analyzing more than one-time difference of the codon usage frequency of *psbA* sequences, the third position nucleotides of preferred codons for *Synechococcus* are all G and C, while most Synechomyoviral sequences (72.7%) had A and T at the third position of their preferred codons.

GeoChip-based comparison of free-living microbial functional gene in *Akashiwo sanguinea* bloom area and control area

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Phytoplankton blooms are a special phenomenon in aquatic systems and occur frequently in coastal waters. Blooms can greatly affect the functional processes in aquatic systems, but comprehensive study of their influences on ecological systems is limited. In this study, a high-through put microarray based technique (GeoChip) was used in order to investigate the response of free-living microbial functional genes to an *Akashiwo sanguinea* bloom which occurred in Xiamen Sea Area in 2011. Results indicated that the *A. sanguinea* bloom greatly changed the free-living microbial functional gene community and the gene structure was more sensitive to blooms than diversity; blooms led to functional gene structure assimilation; the gene diversity was significantly increased and evenness significantly decreased when algal density reached a peak; C, N, P and S cycles, stress, soil-borne pathogens, organic remediation, and energy process related genes were greatly changed; and blooms with high algal density promoted microbial processes, especially C and N cycling, the presence of fungi and virus related genes, and the transferring process from marine to atmosphere.

Stochasticity, succession and environmental perturbations in fluidic ecosystems

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Both deterministic and stochastic factors play important roles in shaping the community composition and structure, but very little is known about the mechanism controlling ecological

succession, especially in microbial communities. To understand the relative importance of stochastic and deterministic processes in mediating microbial community succession and their contributions to ecosystem functioning, here we analyzed the response of microbial communities to environmental perturbations in succession with bioreactor and groundwater samples, which represents controlled and natural fluidic ecosystems, respectively using GeoChip hybridization data. The bioreactor experiment was operated under identical environmental conditions with the same community from wastewater; our results revealed that ecological drift (i.e., initial stochastic colonization) and subsequent biotic interactions created dramatically different communities with little overlap among 14 identical bioreactors, indicating that stochastic assembly played dominant roles in determining the microbial community structure. Neutral community modeling analysis revealed that deterministic factors also played significant roles in shaping the microbial community structure in these reactors. Most importantly, the newly formed communities differed substantially in community functions (e.g. H_2 production) with strong linkages to the community structure. The groundwater microbial communities diverged substantially after external nutrient addition, but subsequently converged to new community structures very similar to, but distinct from the initial states, indicating the resilience and adaptation of the groundwater microbial communities. Consistent with the bioreactor experiment, null model analysis revealed that the community succession was primarily controlled by stochastic rather than deterministic processes in response to environmental perturbations. To our knowledge, this is the first study to explicitly demonstrate the resilience of microbial communities in both controlled and natural ecosystems and the importance of stochastic processes in mediating ecological succession. Elucidating the mechanisms controlling community structure, succession and resilience is fundamental to biodiversity preservation, ecosystem restoration and environmental management.